INFECTION, IMMUNITY
AND
INFLAMMATION

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A STUDY OF THE PHENOMENA OF HYPERSENSITIVENESS AND TOLERANCE, AND THEIR RELATIONSHIP TO THE CLINICAL STUDY, PROPHYLAXIS, AND TREATMENT OF DISEASE

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ST. LOUIS

THE C. V. MOSBY COMPANY

1924

Printed in U. S. A.

Press of
The C. V. Mosby Company
St. Louis

TO

MY FATHER

DAVID F. GURD, M.D.

THIS BOOK IS AFFECTIONATELY
DEDICATED

PREFACE

Since the discovery of the phenomenon of anaphylaxis in 1906, the attention of immunologists throughout the world has been directed in large measure to the study of hypersensitiveness and its relationship to other immunologic processes. Although this chapter in our investigation of disease is not yet fully written, I am of the opinion that there has now accumulated a sufficient amount of data regarding the phenomenon of tissue hypersensitiveness to the presence of foreign albuminous substances, and the companion state, in consequence of which the tissues are rendered insusceptible to symptoms of tissue irritation, to which the name "tolerance" is applied in this volume, to justify a more general knowledge of these facts by the practicing physician and surgeon.

Although I believe that the immunologic process, at least as it applies to the tissue reaction against colloidal proteins, is, in fact, an exhibition of digestive activity on the part of the tissue cells, I do not wish to obscure, by the discussion of the nature of the reaction which occurs between antigen and antibody, the importance of the truth that hypersensitiveness and tolerance are phenomena which are readily demonstrated both in animals and in man. An appreciation of the fact that the two states may, and often do, exist in the same individual is of the utmost importance, both in the study of clinical phenomena and in the treatment and prophylaxis of infectious disease.

Anaphylaxis constitutes the first stage in the immunologic reaction, and, although, under very exceptional circumstances, it may constitute a danger to the life of the animal or individual, it serves a useful purpose in that, in consequence of hypersensitiveness of the tissues to the complex protein molecules which constitute bacteria, the bacterial cell bodies are immediately recognized by the tissue, as irritants. The nor-

mal response of the tissues to the presence of small particles of irritant substances is focal hyperemia, accumulation of leucocytes, and phagocytosis of the irritant particles.

A proper appreciation of the allergic phenomenon, as described by von Pirquet, and its relationship to anaphylaxis and tolerance, places the prevention and treatment of infection by means of bacterial or other protein preparations upon a rational basis. In the absence of a working hypothesis, the production and control of alterations in the immunologic state of the human individual become, not only difficult, but dangerous.

Since 1914, when I first brought forward the point of view expressed in this volume, the clinical study of infectious processes and the control and guidance of inflammation, whether by means of mechanical (operative) or specific immunologic methods, has confirmed, in my opinion, the accuracy of the hypothesis which is elaborated in this contribution.

No attempt has been made to include in this volume a complete bibliography. Specific references have been made only to such publications as have been employed to illustrate a definite phenomenon, or support an hypothesis. My own views have been indicated, and, where it was thought necessary, the contrary opinions have been presented, although I have made it my aim to eliminate unnecessary argument.

Although the general subjects of infection and immunity are considered in a broad way, I have endeavored to limit the discussion to such facts and theories as are of importance to the clinical practitioner. Bacteriology is not discussed from the viewpoint of differentiation of strains of microorganisms, but a study is made of those characteristics of bacteria which determine their pathogenicity and their power to stimulate reactive phenomena on the part of the host.

In the chapters upon the application of immunity principles to the treatment of disease, and in the explanation of disease phenomena, the importance of immunology is indicated, although no attempt has been made to make the presentation of these aspects of the subject exhaustive. Serology, insofar

as it refers to technical methods for the identification of immune bodies, has been excluded. There are at the present time a number of very excellent volumes written in English, in which technical methods of serology are described and the relative importance of *in vitro* reactions discussed. For the presentation of the subject for the laboratory worker, the reader is referred to contributions of this nature. The author has made use of the following works with considerable freedom: Kolmer: *Infection Immunity and Specific Therapy.* Zinsser: *Infection and Resistance.* Wells: *Chemical Pathology,* and *Physiological Reviews,* 1921, Vol. 1, No. 1: The Present Status of Anaphylaxis. Karsner and Ecker: *Principles of Immunology.* Vaughan: *Protein Split Products.* Besredka (Gloyne): *Anaphylaxis and Antianaphylaxis.*

If this small volume, which has been written by a practicing surgeon who, for many years, has made the study of infection and the reaction of the tissues to irritants his special interest and hobby, be of value in stimulating other clinical observers to further inquiry into this fascinating and practical branch of biology, the effort entailed in its preparation shall have been adequately rewarded.

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CHAPTER I

DEFENSIVE AND OFFENSIVE REACTION OF THE BODY AGAINST IRRITANTS

General Introduction

Throughout the lifetime of the individual, the animal or human body is periodically exposed to the entrance of foreign irritant substances. It is only as a result of constant preparedness on the part of the tissues, that the latter are able to maintain their capacity for functioning and the body to prolong its existence.

Broadly speaking, there are two methods by which the offensive and defensive functions of the tissues are accomplished: (1) by the elaboration, on the part of the tissue cells, of soluble substances which are capable of neutralizing or destroying injurious agents; (2) by the phagocytic and lytic activity of certain of the body cells.

Since the study of these two processes requires special technical methods, and hence special training, they have been divided into two groups, both of which are worthy of, and have received by numerous investigators, most careful observation and research. The science which treats of the constitutional reaction of the body to irritants, as evidenced by the elaboration of certain soluble substances and their discharge into the blood, lymph, and other body fluids, is known as *immunology*.

The reactive processes, as manifested by morphologic (visible) changes at the site of the introduction of, or invasion by, irritating substances are termed *inflammation*. Inflamma-

tion consists, therefore, of the focal reaction of the tissues against irritants.

The larger proportion of the cells which take part in local inflammatory reactions are derived from the blood stream, into which they are discharged from the myelogenous and lymphogenous tissues, in increasing numbers, during the course of reactive phenomena. There occur during the course of infectious diseases changes in the productive activity of the tissues, more particularly of the bone marrow, as well as deviations from the normal cell content of the blood. This readjustment of blood cell precentages may be conveniently called the *hemocellular reaction.*

All forms of reaction, with the exception of rare instances of reflex reaction, are stimulated by the presence in the tissues, in which the reaction occurs, of some form of irritant. If we attempt to divide the causative factors which provoke inflammatory reactions according to their physical nature, we find that in so doing, we understand, to a considerable degree, the usefulness of the individual elements taking part in the reactionary process, and, thus, are enabled to appreciate the rationale of the reaction itself.

Three main types of irritant are met with, viz.:

(a) Diffusible substances, such as toxins, alkaloids, crystalloids.

(b) Colloidal substances, proteids. These may be either (1) soluble, as egg albumen and serum protein, or (2) particulate, as bacterial cells.

(c) Insoluble and nonfermentable substances, such as carbon and the metallic elements.

As might be expected the manifold substances belonging to any group vary much in toxicity or irritant qualities; it may be noted however, that no matter what may be the relative toxicity of the foreign substance, an attempt is usually made by the tissues of the host to combat this effect and to remove the source of irritation. It will be observed, moreover, that the physical as well as the chemical nature of the

foreign substance determines the type of reactionary process exhibited.

The pathogenic effect of irritants upon the body tissue is due to a variety of injurious actions:

1. By their simple mechanical presence foreign substances, even though not in themselves irritating to the tissues, may destroy cells in their immediate vicinity and interfere with the function of the part in which they are situated, e.g., drainage tubes, projectiles, anthracite pigment, and air and other gases (such as are produced by *B. aerogenes capsulatus* during its growth).

2. If the irritant substance be soluble, it is carried to distant parts of the body and may cause injury to cells far removed from its point of entrance, e.g., bacterial and other toxins, and alkaloidal poisons.

3. Insoluble toxic substances may injure tissue cells in juxtaposition to them.

4. Living, growing bacteria and animalculae may, in addition, destroy tissue as the result of their metabolic activity, e.g., muscle tissue by the *B. aerogenes capsulatus,* and red blood cells by the plasmodium of malaria.

Irritants not only differ in physical and biological type, but the extent of their injurious action varies to a very considerable degree, some being almost inert, e.g., catgut and most of the metallic elements; whereas others (hydrocyanic acid, tetanus toxin) are so potent that extremely small amounts lead to rapid and violent death of the individual.

As we study the reactive changes which occur in the animal body, we find, as might well be expected, that different types and degrees of irritants are met by the exhibition, on the part of the body tissues, of different methods of defense. We note, moreover, that certain substances are not antagonized in their action in any way and that the fate of the individual into whose tissues the irritant gains entrance will depend largely, or entirely, upon the amount of the substance introduced. Such we find to be the case with the alkaloidal poisons, as well as the majority of toxic mineral substances, such as arsenic,

lead, and mercury. Experiments prove that repeated introduction into the animal body of certain drugs, such as the aldehydes and alcohols, as well as a number of alkaloids, is followed by the exhibition of a certain degree of tolerance to such drugs. Drug tolerance of this sort is not looked upon as an immunity reaction; nor is there any suggestion that this form of tolerance is related to tolerance to the presence of protein derivatives in the tissues.

It is pointed out later in this volume that the most important type of irritant and the one which is met by specific antibody production is that composed of protein complexes in colloidal form. Although all colloids are not antigenic,[1] it is a fact that, so far as we know at present, all antigens are colloids. The chief obstacle to a more adequate understanding of the defensive reactions of the tissues is due to the fact that the chemistry of the colloids is as yet obscure.

Since bacteria and their derivatives constitute by far the most important group of irritant substances, they have been made the object of special consideration in this volume. The action of microorganisms as causative factors in disease is complicated by the fact that, in studying them, we are dealing with a type of irritant, the concentration of which fluctuates, and the chemical constitution of which is very insufficiently known.

During the earlier studies of immunity processes, bacteria were employed almost exclusively as antigens. Later the data obtained as a result of such experiments were generalized so as to be applicable to all antigens. Since the discovery of the anaphylactic reaction, the fundamental observations have been made with nonbacterial antigens.

Serum Reactions

There are present in the body fluids, blood, lymph, etc., substances which are capable of combining with, digesting, or absorbing, specific chemical bodies, and which are known

[1]Any substance which stimulates the tissue cells to produce antibodies is called an antigen.

as antibodies. The study of the nature, method of production, and manner of action of such antibodies, is known as immunology. Substances against which specific antibodies are produced are called *antigens*.

The science of immunology is recognized today as one of the most important of all biologic branches of medicine, since it attempts to discover and explain the basic principles underlying protection from, and cure of, infectious diseases.[2]

In this chapter, it is not my intention to discuss in detail, the nature of such antibodies, nor their method of production, but rather to draw attention, in a broad way, to the important part which they play in disease conditions. At present it is sufficient to state that the body fluids of man and animals normally contain bodies which are able to act as antidotes to many forms of toxins, and also substances which are able to alter certain solid (particulate) organic materials, notably proteins, in a manner which leads to their inactivation as harmful agencies and their subsequent destruction. It must be noted also, that, to a very considerable extent, the toxin or foreign material is neutralized by a *specific* substance present in the blood, and, furthermore, that under certain circumstances of stimulation there is exhibited by the body, the ability to manufacture an enormous increase in the number of such specific antibodies.

An individual, or animal, whose tissues contain substances which lead to the immediate overwhelming of the invader, is protected against infection. The state of the body, by virtue of which it is able adequately to defend itself, is termed *prophylaxis*.

There is a natural prophylactic state against certain types of bacteria, which is known as *natural immunity*. In general, however, the immune state is developed only after the stimulation of the tissue cells, by previous successful encounter with like germs, *active immunity*, or by the introduction of specific

[2]In addition, immunologic contributions of recent years have shown that a number of previously obscure clinical entities, e. g., asthma, hay fever, and eczema, are the result of the reaction of the tissues to the presence of nonviable protein antigens.

antibodies contained in the serum of some other individual or animal, in which active immunization has previously been stimulated. The gift of antibodies in this manner, is known as *passive immunity.*

For the most part, the production of antibodies under stimulation is specific; that is to say, the activity of the antisubstances elaborated by the tissues, and discharged into the body fluids, is directed chiefly against the antigen, whether bacterial toxin, heterologous protein, or cell body, which has stimulated their production.

Cellular Reaction

In the defense of the tissues against invasion by bacteria, not only are there chemical substances produced, the action of which is inimical to the life of the microorganisms, but certain cellular and tissue constituents of the body take part in the destruction of the bacterial cell by a process of ingestion and subsequent intracellular dissolution of the bacterium. Such a process is known as *phagocytosis.*

In a general way, we note that certain foreign substances stimulate phagocytosis on the part of special types of cells. Exactly what the attributes of the foreign substances must be, in order that the cells may be induced to attempt their destruction, is insufficiently understood. It is evident, however, that it is because the foreign material is irritating, and hence injurious, to the tissues that this means is adopted for its destruction. The type of cell, which takes part in protective reactions, is determined largely by the nature of the irritant substances. As a general rule, markedly irritant foreign substances are the object of attack by the neutrophile polymorphonuclear leucocyte.

It may be stated that it would appear that the endolysins of certain body cells, notably the leucocytes (leucoprotease), are considerably more potent than the serum lysins.[3] The exhibition of phagocytic activity on the part of the cells is

[3]Lysins—Substances potent to cause solution of proteins in particulate form.

thus the more effective, as well as the more economical, means of combating infection.

If microorganisms which gain entrance to the body tissues are able to adapt themselves to the conditions as regard oxygen supply, moisture, and foodstuffs—qualities which all parasitic, and many saprophytic, bacteria possess—their continued growth will depend upon the adequacy of the defensive properties at the disposal of the body.

If the combined antibody and phagocytic properties of the tissues be sufficiently potent, the invader is forthwith destroyed; no infection occurs; and no visible pathologic lesion is manifest. This phenomenon, which may be termed *subinfection* (Adami), is in itself harmless, and is in reality useful, as it stimulates the production of larger numbers of antisubstances.

If the number of microorganisms introduced, considered as units, be larger than the number of available units of antibacterial properties, not all of the former are destroyed, and the survivors which maintain their vitality continue to proliferate. If the capacity for antibody production be well developed, the body at once commences the manufacture of new antibodies, with the result that, although the number of units of bacteria is being rapidly multiplied and hence large numbers of antibody units are exhausted or inactivated, there eventually comes a time when the rate of production of antibacterial properties surpasses that of the multiplication of bacteria. From this instant the number of bacteria present in the tissues commences to diminish, with the result that ultimately the invaders, or at least those brought in contact with the blood stream and body fluids, are completely destroyed.

CHAPTER II

INFECTION AND INFECTIOUS AGENTS

Introduction

Infection consists in the entrance into, and growth within, the animal (or plant) tissues of minute living bodies of vegetable or animal origin. The process or state of infection has been differently defined by various writers. Adami, and others, consider infection to comprise the succession of changes induced in the organism, generally, by the growth within it of microbes. Such a definition embodies not only the phenomenon of infection but also the reaction on the part of the tissue against invasion by the morbific agent; for, although it is true that, as a rule, unusual cell accumulations and immune body production follow the entrance of bacteria, protozoa, etc., into the tissues, these changes represent reactions on the part of the host, and are better studied under the headings of immunity and inflammation.

Infective agents may be divided into four main groups, as follows:

I. Bacteria.
II. Yeasts, moulds and fungi.
III. Protozoa and metazoa.
IV. Filterable viruses.

Bacteria, as a class, are commonly considered to belong to the lowest type of plant life and are closely related to, and are indeed in many instances only with difficulty differentiated from, the more simple moulds and fungi.

The *protozoa* represent the simplest form of animalculæ. Among the higher animal forms (*metazoa*) the helminthes (worms) are the most important. Their activity is usually confined to the intestinal tract.

The vegetable and animal types mentioned are readily demonstrated visually and, with few exceptions, have been studied by means of animal experiment, or under conditions of cultivation *in vitro*. There remain a number of infective bodies whose potency in the production of disease and the induction of immunity are well established, but which, owing to their minute size, are invisible under the microscope. Since these bodies are capable of filtration through the finest porcelain filters they have been termed *filterable viruses*.

Practically all local pathologic lesions which are classified as inflammations, as well as many constitutional diseases, are caused by the presence, and growth, in the body of one or the other of these microscopic vegetable forms (bacteria, yeast, and moulds) or of those animalculæ known as protozoa. No aspect of the study of disease is of so great importance to the practicing physician or surgeon, as an appreciation of the mode of action of bacteria in producing pathologic changes in the body, and the various means at the disposal of the body, by which it may overcome such invaders.

The different manifestations of infection, as recognized and classified clinically, represent chiefly the evidences of the reaction of the tissues of the host under stimulation by the invader. It has been found, moreover, that infection by different types and species of microorganisms, is, in many instances, followed by reactions of a specific type, and, since many pathogenic infective agents demonstrate a predilection for certain particular tissues, it is possible to recognize from the clinical manifestations alone the nature of many infecting microorganisms. Thus are diagnosed infection by the typhoid bacillus, and by the streptococcus in erysipelas, as well as invasion by the viruses of smallpox, measles, and other exanthemata.

There is a comparatively large number of affections the specific etiologic agents of which cannot be thus recognized and for whose diagnosis bacteriologic methods must be employed. The more precise methods of the laboratory permit, moreover, an earlier, as well as a more exact diagnosis than

those of the clinician. In certain diseases, as in syphilis, the identification of the specific infective agent permits the employment of proper treatment, at an earlier period in the course of the disease, than would otherwise be possible.

Historical

The history of the development of the biologic science of bacteriology, as applied to the observation and treatment of disease, is of comparatively recent growth; for, although, even as far back as 1683, Leouwenhoek, a lens maker of Holland, described minute moving forms in material collected from the mouth, and, although, from time to time, observations were recorded in which more exact details of the structure of such forms were mentioned (Müller, 1786, Ehrenberg, 1838), it was not until the early part of the latter half of the last century that Pasteur, although investigating as a chemist the cause of deterioration of certain wines, recognized and described the all-important part played by bacteria in bringing about changes in the chemical nature of their environment. Coincidently with the acquirement of this new knowledge Pasteur applied it to the study of pathologic conditions, and to the production of immunity.

Up to the time of Koch's employment of solid culture media in the isolation of single pure strains of microorganisms, the study of the nature and properties of bacteria was accomplished only with the most tedious labor, and was rewarded with relatively meager results. From this time, 1876-1881, however, the possibilities of more precise methods of study by means of the employment of pure cultures were appreciated. Gradually, but with steady progress, a constantly increasing number of pathogenic processes have been proved to be due to bacterial activity, or to the presence of fungi or protozoa.

It is to Koch, moreover, that we owe the promulgation of a series of postulates which have been accepted as being useful and necessary in associating a given microorganism with a specific disease. According to Koch's postulates the chain of evidence upon which this connection may be based, is:

(1) The demonstration of the microorganism in all cases of the disease, in such a relationship to the pathologic process that its causative nature seems probable: (2) the artificial cultivation of the organism in pure growth: and (3) the reproduction of disease by inoculation of the microorganism so isolated.

Although, previous to 1860, the subject of bacteriology, as such, was practically unknown to all but a very occasional worker, the fact that there was some substance, or virus, which was responsible for the development of disease processes in man, was appreciated, as is evidenced by the employment by Jenner of the exudate from cases of vaccinia to inoculate the healthy individual. In obstetrics also, White, Semmelweis, and Holmes recognized that as the result of uncleanliness, contact with infected patients, etc., cases of puerperal fever developed. Basing their actions upon these observations, they adopted methods which were strikingly modern in their nature, and which were successful in controlling markedly the incidence of this disease.

Following the appreciation of bacteria as the cause of wound infection, Lord Lister (1865) applied his knowledge of the germicidal action of certain substances, especially carbolic acid, to surgery. From this time dates the advance in surgical technic with which we are all familiar; for, although a more comprehensive recognition of the biology of bacteria has led to the discarding of some of the practices thought by Lord Lister to be necessary, the value of his work cannot be overestimated.

There are several aspects of the biology on life history of bacteria, which it is of the greatest importance that the practicing physician rightly appreciate. For instance, an adequate idea of the various factors which influence favorably, or the opposite, the life and multiplication of microorganisms, is of the greatest value, not only in the proper handling of infectious processes, but also in prophylaxis. Again the chemical constitution of bacteria is of paramount importance, since their activity as pathogenic agents, as well as their protection

from the defensive properties at the disposal of the body, depend, in large measure, upon their more intricate chemical peculiarities. An attempt will be made to prove in consideration of immunology in a later chapter, that it is largely upon the relative amount and exact nature of the protein constituents of the bacterial cell body, that the various clinical manifestations of bacterial invasion (infection) depend.

One of the most important contributions to our knowledge of the method of action of chemical germicides, as also of immune processes, is that of Ehrlich in his recognition, and elaboration, of the principle of chemotaxis or receptor affinity. According to Ehrlich's hypothesis, the ability of any living cell to incorporate into its protoplasm chemical substances, present in its vicinity, depends upon the presence of receptors, or special unsatisfied molecules, which are capable of attracting and combining with specific substances. The normal metabolism of the cell depends upon the presence of such receptors for useful and essential food particles. Ehrlich proved that not only do cells possess affinities for useful and helpful foreign molecules, but that they also possess receptors capable of attracting and combining with injurious substances which may ultimately lead to the death of the cell.

Morphologic and Chemical Characteristics of Bacteria

Structure.—Bacteria consist of unicellular organisms which exhibit an extremely simple structure. In contrast to the more complicated morphology of higher vegetable, as well as animal, cells, bacterial cells show no differentiation into a distinct nucleus and cytoplasm; although it is probable that infinitely small masses of nucleo-protein (chromatin) material which are scattered throughout the cell body, and which constitute a large part of the total protein content of the bacterial cell, influence the life processes of the bacterium in a manner similar to that exerted by the nucleus of higher types of cells. It must be regarded as possible, however, that there may be a specially differentiated portion of the cell represent-

ing the nucleus which, so far, has not been demonstrated by the technical means at present at our disposal.

The majority of bacteria consists simply of a homogenous material surrounded by a cell membrane[1] and in which are scattered the chromatin granules or network just mentioned; others contain other substances which can be tinctorially differentiated and which are known as *metachromatic granules*. The function of the latter is, at present, not understood.

Other bacteria, notably the tubercle bacillus and allied groups (*B. leprae, B. smegma, etc.*), are enveloped in a layer of fatty or waxy material which gives to them their so-called *acid-fast* properties. The protective effect of the capsule makes it difficult to stain the cell protoplasm, and on the other hand if powerful stains be employed, permits the body to resist the decolorizing action of dilute acids and alcohol.[2] Certain bacteria (e.g., *B. aerogenes capsulatus, B. mucosus capsulatus, pneumococcus and streptococcus mucosus*) produce, and surround themselves with, a colloidal material similar to mucin, which is known as a *capsule* and which by the employment of special tinctorial methods can be readily demonstrated.

Size.—Bacteria, as a class, consist individually of extremely small masses of protoplasmic material in which appear chromatic granules or networks; they are rendered visible only by the use of high power objectives. The individual cells vary somewhat in size, measuring from 0.1 to 0.6 microns in diameter, and from 0.8 to 10.0 microns, or greater, in length. If these measurements be compared with that of the human red blood cell, 7.5 microns, the diminutiveness of these troublesome parasites is more easily appreciated. Since different types of bacteria vary much in size, it is impossible to accurately determine the weight of the individual cell, dried bacteria[3] of the size of the tubercle bacillus (2.0 to 4.0 microns,

[1]The exact nature of the cell membrane has so far not been demonstrated. Vaughan has apparently proved that it is not cellulose, since it gives no carbohydrate reaction.

[2]It is probable that the majority of bacteria possess in the cell covering a small quantity of waxy material, but that this substance is not sufficient in amount to confer "acid-fast" properties upon the bacterium.

[3]Hammerschlag (Centralbl. f. klin. Med., 1891, xii, p. 9) observed that the average water content of the tubercle bacillus is 85.9 per cent.

by 0.3 to 0.5 microns) weight each, upon the average, about 1/12,000,000,000 mg.

Since the potency of bacterial vaccines in the production or guidance of immunologic processes is dependent, almost exclusively, on the amount of antigenic protein in the bacterial cell body, it is obvious that the fact that bacteria vary greatly in size and weight is of great importance in gauging the probable dose (in number of microorganisms) which must be employed.

Spore Formation.—Certain bacteria have the property of protecting themselves from extinction under unfavorable circumstances, by means of the development of resting, nonvegetative, highly resistant forms, known as "spores." The pathogenic bacteria which possess this means of perpetuation are the *B. anthracis, B. aerogenes capsulatus, the B. tetani.* There is also a large group of spore-bearing saprophytes and facultative parasites included in the *subtilis-mesentericus-proteus* groups.

Since bacterial forms possess such very different properties of resistance to outside influences, it is customary to speak of actively growing spore-forming bacteria, as well as all those which do not form spores, as vegetative forms. It is noteworthy in this connection that bacteria, capable of spore formation, do not usually produce such forms if conditions be favorable for their growth. This fact is of the utmost importance in the employment of intermittent methods of sterilization.[4]

For the surgeon the formation of spores, upon the part of certain bacteria, is of special interest. The ordinary means adopted for the destruction of vegetative bacteria are useless in destroying the vitality of spores. Thus boiling does not injure them, nor does prolonged drying result in devitalization.

[4]Spore-bearing bacteria are destroyed by temperature of about 100° C. only if they be in a vegetative state. If circumstances (e.g., temperature, foodstuffs, oxygen supply) are suitable during the interval between exposure to heat, all spores are so influenced that they are replaced by actively growing forms, nor are fresh spores formed. Under these conditions, sterilization of infected media and solutions for intravenous or subcutaneous medication, may be accomplished.

Tinctorial Differentiation.—One of the most important means for differentiating microorganisms is by their staining reactions, or affinity, for certain aniline dyes. By far the most important of such methods is that of Gram. The principle of this staining method is based upon the fact that certain bacteria when stained by aniline-methyl or aniline-gentian-violet, and subsequently treated with a watery iodine solution,—Lugol's solution—do not decolorize when treated with 95 per cent absolute alcohol. Such bacteria are termed Gram-positive, whereas those which are not resistant to alcohol in this manner are classified as Gram-negative.

The resistance of a bacterium to decolorization by Gram's method is, in a general way, comparable to its resistance to lysis or destruction by antibodies produced by the tissues.[5] Those bacilli which decolorize by Gram's technic are subject to dissolution when acted upon by fresh immune serum, whereas the Gram-positive cocci are but little injured, either morphologically or biologically, by soluble antibodies.

Among pathogenic bacteria all cocci, with the exception of the gonococcus (*Micrococcus gonorrheae*), meningococcus (*Micrococcus intracellularis meningitidis*), *Micrococcus militensis,* and the *Micrococcus catarrhalis,* are Gram-positive; whereas all pathologic bacilli, with the exception of the *B. diphtheriae,*[6] *B. aerogenes capsulatus, B. anthracis,* and the acid-fast bacilli (tuberculosis, leprae), are Gram-negative. *B. tetani,* and *B. mucosus capsulatus* (Friedländer's pneumobacillus) are variable, sometimes retaining and sometimes giving up the primary stain.

Characteristics of Bacteria Determining Their Pathogenicity

In order to understand the results of infection of the tissues by bacteria, it is necessary that the manner of vicious action on the part of bacteria be appreciated. "Bacteria are important on account of the changes which they bring about

[5]It may be assumed that the resistance of Gram-positive bacteria to staining, to decolorization and to lysis is dependent upon relative impermeability of the ectoplasm or covering of the cell body.

[6]Also B xerosis and B. Hoffmani, the so-called pseudodiphtheria group or diphtheroids.

in the chemical nature of their environment'' (Jordan). Bacteria lead to local death of tissues and to constitutional toxemia and induce reactive processes (inflammation) in four separate ways, which depend upon (1) the production of specific and diffusible toxins; these act both when bacteria are extracellular and when they are within the tissue cells themselves (true toxins or exotoxins); (2) the presence as part of the bacterial cell, of protein bodies which, under the influence of substances present in the body fluid of infected animals, are altered with the liberation of toxic end products (secondary toxins, endotoxins, anaphylatoxins); (3) the production of toxic alkaloidal substances known as ptomains,—cholin, cadaverin, etc.,—through the breaking down of tissue cells as the result of the metabolic activity of the bacteria; (4) mechanically, also, bacteria may impair tissue nutrition through occlusion of terminal arterioles by bacterial cell masses.

From a biologic standpoint these substances, toxins, endotoxins, ptomains, as pointed out by Jordan, consist of (1) secretions, that is, those substances which subserve some purposeful end in the cell economy; these may be retained within the cell or may pass into the surrounding medium; (2) excretions—those substances which are expelled because useless to the organism; (3) disintegration products, which result from the breaking down, fermentation and decomposition of food substances. These substances, which are among the most important, depend upon the enzyme activity of certain of the secretions; (4) the true cell substances (endotoxin, anaphylatoxin—See ''Immunity'').

As is readily appreciated, the manner of production and relationship of the various substances to the physiologic processes of the bacterial cell are of comparatively little importance as compared with the physical and chemical characteristics of the substances themselves.

As is the case with the higher vegetable and animal cells, the changes in the chemical nature of their environment, on account of which both the useful and certain of the injurious activities of bacteria depend, are due to the elaboration of

soluble ferments or enzymes. Certain of these, as, for instance, the gelatin-liquefying enzyme of most saprophytes and a certain number of parasitic bacteria, diffuse out of the cell, whereas others act only upon assimilated simple or complex foodstuffs.

Certain bacteria such as the tetanus and diphtheria bacilli, which possess to a very limited extent the capacity for proliferating in normal tissues, secrete an extremely powerful poison. It is clear, says Zinsser, that unless these bacteria produced very powerful poisons, they would not be pathogenic at all, and would not be brought to our attention in connection with human disease.

It is indeed, he says, quite conceivable that there may be a great many bacteria as little invasive, and which may produce true toxins of less potency; these would never functionate as pathogens, simply because the weakness of their poisons, and their lack of invasive power, taken together, render them entirely incapable of establishing a foothold in, or upon, the living body. On the other hand, it is quite conceivable that bacteria which possess the property of invasiveness to a high degree, need not produce poison of any great potency, in order to cause symptoms of toxemia in the invaded animal body.

Toxins.—But few types of bacteria depend for their pathogenicity upon toxins of the first class, namely, soluble and diffusible toxins which are readily isolated from the bacteria themselves, although a large number do develop toxic substances of mild potency. Of those whose action depends chiefly upon soluble toxin elaboration, the B. diphtheriae, B. tetani, and B. botulinus, are by far the most important. Streptococci and pneumococci produce leucocyte poisons (leucocidin) as well as hemolysin; these properties are, however, of relatively little importance when compared with the extreme virulence of these bacteria. In addition to the bacteria mentioned above, the dysentery, plague, and pyocyaneus bacilli and the cholera vibrios all produce moderately active toxins.

Bacterial toxins manifest a selective action upon certain tissue cells. Tetanus toxin, diphtheria toxin, and the pyocyaneus toxin, as well as the filtrable virus of rabies, attack the central nervous system. The vagus is affected by the toxins of the diphtheria bacillus, the influenza bacillus and the B. pyocyaneus. Rainy[7] found distinct biologic changes in the motor cells in patients dying from diphtheria intoxication.

The majority of soluble toxins produce a peripheral vascular dilatation and thus induce lowering of blood pressure.

The special affinity of toxins for certain tissues is well exemplified by the tetanus toxin which produces its effect through its action upon the central nervous system. If an animal be injected intravenously with tetanus toxin, and the blood examined at the end of three or four minutes, no toxin can be demonstrated in the blood; it can, however, be recovered by a process of extraction from all tissues but the central nervous system, and this, notwithstanding the fact that it acts obviously through its effect upon the central nervous system.

If one gram of guinea pig brain is triturated with 10 cubic centimeters of normal salt solution an emulsion is made which is capable of neutralizing 100 fatal doses of the toxin (for white mice). On the other hand emulsions of other tissues, blood, spleen, muscles, etc., do not possess this property (Ransom). It is, then, evident that as a result of its affinity for the toxic body the central nervous system renders itself susceptible to its poisonous action.

Although these facts are true of most animals, white mice, guinea pigs, etc., it is found that in the rabbit both liver and spleen have the property of fixing tetanus toxin and thus mitigating the effects of its injection. Again, in the tortoise no fixation of the toxin in the central nervous system or in other tissues occurs, neither is this reptile subject to tetanic intoxication.

True toxins are recognized to be catabolic products of cell metabolism,—bacterial or higher vegetable; they act in ex-

[7]Rainy: Jour. Path., 1900, vi, 444.

tremely minute doses; 0.0002 milligram of diphtheria toxin is sufficient to kill a guinea pig weighing 250 grams, in three days. Specimens of tetanus toxin have been prepared that are fatal to white mice in doses as small as 0.0005 milligrams. When toxins are injected into animals in sublethal doses, antitoxin is produced by the tissues of the animal.

Although toxins have not been isolated in a pure state, they are believed to be proteins. They diffuse with difficulty, thus indicating their colloidal nature. Amino-acids are not derived by hydrolysis. They give a positive biuret and a negative Millon's test, thus showing their protein constitution and the absence of aromatic radicles.

Diphtheria toxin is precipitated by alcohol and by full saturation with ammonium sulphate. It is destroyed promptly by boiling and by exposure to a temperature of 73° C. for five minutes. It is injured by freezing and by the action of light.

The amount of toxin produced by one and the same strain of microorganism is tremendously influenced by the culture medium in which its multiplication takes place. Kendall has drawn attention to the fact that in the presence of a liberal supply of carbohydrate foodstuffs, the majority of bacteria will not attack protein substances, except to the limited extent necessary for their vital processes. Theobald Smith has shown that the diphtheria bacillus produces toxin, only when forced, as the result of an absence of carbohydrates, to subsist more or less exclusively upon nitrogenous material. The same holds true of the tetanus bacillus, with regard to toxin production by that bacterium.

Ptomains.—Although all pathogenic bacteria live upon, and utilize, body tissue to supply nutrition for their metabolic activities, a small percentage only, liberate or synthesize from the tissue proteins poisonous substances of any special toxicity. A certain number, however, more especially the anaerobic putrefactive bacteria,—B. aerogenes capsulatus, and numerous saprophytes, as well as many of the facultative parasites including the colon bacillus,—decompose the body tissues with

the formation of highly toxic substances which can be isolated in crystalline form. These substances, known as ptomains (Brieger), are alkaloidal in nature and are represented by cadaverin and cholin. The action of many of these poisons is very powerful and, since no immunity is established against them, individuals infected by such microorganisms soon suffer from an intense toxemia, designated by the term sapremia, which, characteristically, unless the focal proliferation of bacteria be controlled, soon results in death of the host.

Although ptomains occasionally cause disease and sometimes even death, these substances are gradually being relegated to a much less important place in our appreciation of pathologic processes, than they have hitherto held. Especially is this the case, since it has been recognized that the majority, at least, of cases of so-called food poisoning, are the result of the activity of bacillus botulinus, or microorganisms of the paratyphoid group.

Effects of Environment upon Toxicity.—In addition to the properties, mentioned in the foregoing section, which are exhibited by bacteria in order that they may be able to gain, and maintain, a foothold in the tissues, other qualities deserve our attention. It has been previously stated that in addition to catabolic and anabolic toxic substances such as the true toxins, ptomains, etc., the bacterial cell body is, under certain conditions of the tissues of the host, of very actively toxic potency. In a subsequent chapter it is shown that this secondary or anaphylactic toxicity of microorganisms is dependent directly upon the protein content of the bacterial cell. It has, furthermore, been proved that certain bacteria liberate varying quantities and qualities of soluble bodies—indol, skatol, aromatic oxyacids, lactic acid, and acetic and succinic acids, etc., certain of which are injurious to the tissues. The bacterial cell itself is found to possess a different chemical constitution under variable conditions of environment.

"Lyons and Cramer[8] have analyzed bacteria grown upon

[8]Lyons and Cramer: Quoted by Kendall, Jour. Med. Research, 1911-12, xxv, 117.

media with and without carbohydrate, and, have made the very interesting and important observation that the actual chemical composition varies, the nitrogen content being greater in those organisms grown in media containing no carbohydrate, less in media containing carbohydrate. They have furnished definite figures showing in a striking manner just what these differences are. Their figures are fairly in accord, and, inasmuch as the observed differences between the selected media are greatly in excess of the probable source of error, it may be assumed that the difference in composition of the bacteria is proved." (Kendall).

Lyons used three bacilli for his experiments. The accompanying table contains his results.

ORGANISMS		DEXTROSE IN PER CENT		
		1	5	10
	(Nitrogen-substance	62.75	58.88	45.88
Pfeiffer B.	(Ether extract	1.68	3.50	2.67
	(Alcoholic extract	12.17	17.30	29.60
	(Ash	7.16	2.97	3.09
	(Nitrogen-substance	71.81	51.12	46.25
Bacillus No. 28	(Ether extract	3.32	3.84	2.84
	(Alcoholic extract	11.39	15.19	22.78
	(Ash	6.51	3.66	4.18
	(Nitrogen-substance	61.05	44.31	33.25
Thread Bacillus	(Ether extract	1.74	2.24	1.87
	(Alcoholic extract	18.40	21.80	27.50
	(Ash	8.09	4.50	3.02

Cramer used four bacilli; his experiments are more extensive than those reported above. His organisms include the following: Pfeiffer's bacillus (1), No. 28 (2), Pneumonia bacillus (3), and the Rhinoscleroma bacillus (4).

The importance of such alteration in the protein content of bacterial cell bodies, under different conditions of environment, may be of great importance in determining the clinical evidence of toxicity of infecting microorganisms.

Brown[9] has published observations upon the B. welchii (B.

[9]Brown: Ann. Report Mass. State Board of Health, 1909.

	NITROGENOUS SUBSTANCE			EXTRACT-ETHER ALCOHOL			ASH		
Bacillus	Peptone Per Cent	Dextrose Per Cent		Peptone Per Cent	Dextrose Per Cent		Peptone Per Cent	Dextrose Per Cent	
Medium No.	1	5	5	1	5	5	1	5	5
	1	2	3	1	2	3	1	2	3
No. 1	66.6	70.0	53.7	17.7	14.63	24.0	12.56	9.10	9.13
No. 2	73.1	79.6	59.0	16.9	17.83	18.4	11.42	7.79	9.20
No. 3	71.7	79.8	63.6	16.3	11.28	22.7	13.94	10.36	7.88
No. 4	68.4	76.2	62.1	11.1	9.06	20.0	13.45	9.33	9.44

aerogenes capsulatus) which are of great import in indicating the importance of foodstuffs in this respect. "When grown in ordinary broth plus tissue and inoculated into grown pigs, this anaerobe produced no lesion beyond a small subcutaneous nodule that was transitory, but if grown in bouillon plus tissue that had been rendered sugar-free by fermentation with B. coli, it was pathogenic for guinea pigs, producing the characteristic lesions of B. aerogenes capsulatus infection."

Bacterial Proteins—Endotoxins—Anaphylatoxins.—Although a certain number of bacteria, notably the diphtheria and tetanus bacilli, produce soluble highly poisonous substances, it can be proved that the manifestations of intoxication which accompany the majority of infectious diseases are not due to such toxic products, but that the injurious substance is derived from the body of the bacterium. Furthermore, it appears that unless partial dissolution, or protein cleavage of the bacterial cell be accomplished, the irritant property of the bacterium is not exhibited.

Two views have been held with reference to the nature of this poisonous moiety. Pfeiffer, who was the first to recognize the presence of such a toxic principle (See Immunity; Endotoxins), considers that the toxin exists, preformed, in the bacterial cytoplasm and that by partial lysis of the cell body it is set free.

The second view, and the one which receives greater support from recent experiments, is that which supposes that as

a result of the interaction of certain specific substances in the body fluids, the albumin molecule of the bacterial cytoplasm is altered so that it becomes an irritant to tissue cells. By a large group of observers it is believed that the alteration of the protein molecule is a partial cleavage or degradation apparently to the stage of peptone formation. This view had received its greatest support from the work of Vaughan and his associates. These observers have been successful in producing *in vitro*, by simple hydrolysis, substances which appear to possess all the properties of Pfeiffer's endotoxins.

In contradistinction to the primary or essential toxins produced by microorganisms, such as B. diphtheriae, this potential toxicity of the protein constituents of the bacterial cell in the manner described, is known as secondary or anaphylactic toxicity. Depending upon the point of view of the author, this irritant has been designated by various names, including toxalbumin, anaphylatoxin (Friedberger), and apotoxin (Richet). This secondary toxicity of bacterial proteins has recently been the subject of much investigation and study, and has proved to be of paramount importance in the appreciation of disease phenomena. In a later chapter (Immunology), is discussed the means whereby this apparently protein-splitting process is accomplished and the conditions under which the splitting property on the part of body fluids is increased or diminished; also the manner of action of the so-called "anaphylatoxins." Suffice to state, at the present time, that the protein content of bacterial cells, although they are inherently nontoxic, may be so acted upon by specific substances in the serum, probably of the nature of ferments, that soluble highly irritant albuminoids are set free.

Zinsser believes that "endotoxins" do not form the matrix of toxic-split products produced in the circulation by the sensitizer-alexin complex, as conceived by Friedberger and others; but injury by their reaction with the fixed tissue cells, as conceived by Vaughan, he says, cannot be excluded.

Zinsser sums up the evidence regarding the endotoxin as follows: "The body substances of most Gram-negative bacteria

are toxic for the ordinary laboratory animals. These toxic properties are common to many nonpathogenic, as well as pathogenic bacteria of this class. It is uncertain, but unlikely, that they are pharmacologically specific. In a large majority of cases these substances have been found to be relatively resistant to heat, and do not deteriorate readily on standing. Such 'endotoxins' do not induce neutralizing antibodies of any marked degree of potency, but they do induce specific protein sensitizers by means of which partial specific neutralization of their efforts may be accomplished. Similar endotoxic substances have not been consistently produced with Gram-positive bacteria."

The simplest explanation of these facts, in the author's opinion, is that autolysis of the Gram-negative bacteria readily occurs *in vitro,* as well as lysis (digestion) *in vivo.* The irritant properties exhibited by the body substance is due to partial cleavage of the protein molecules with exaltation of chemism of the split products. Since the body substance of the Gram-positive bacteria is more resistant to the effects of protein-splitting substances, in their environment, irritant properties are less easily proved for the cytoplasm of this group of microorganisms.

Peptotoxins.—Attention has recently been drawn by several different investigators to the fact that, in addition to the simple or primary toxins, such as characterize the diphtheria and tetanus bacillus and the intracellular protein content of bacteria, which in common with other heterologous proteins, is potent to induce anaphylactic symptoms, there is produced in the medium in which certain bacteria are grown a soluble protein substance which is capable of precipitation, but not of coagulation by absolute alcohol and which is extremely toxic when introduced into sensitized animals, i.e., animals which have previously received injections of the same or a similar preparation. Since this toxic body is produced only when the various bacteria which are capable of producing it are grown upon media containing peptone, it has been termed

by Besredka and Ströbel "peptotoxin."[10] These observers assume tentatively that peptotoxin and anaphylatoxin are probably identical.

Whatever the nature of this poison, it is probably identical with that studied by Zinsser and Parker. It appears to be a fact, in view of the observations of these investigators, that many bacteria, such as the B. influenza, streptococcus, typhoid and dysentery bacilli, produce a substance which may be extracted with ease from the culture medium, either liquid or solid, in or on which they have grown. These toxic substances resemble one another in many ways, qualitatively, no matter what their source. They produce paralytic symptoms in rabbits, after but a short incubation period. They are harmless for guinea pigs; they are not stable, and are destroyed by temperatures of about 80° C. Serologic work has been done to prove the true toxin nature, and specificity of such poison. In general, the sera produced have neutralized the toxic substance to a limited extent only. It is noteworthy, moreover, that such neutralization has been more regularly proved when the poison substance, and the antibody containing serum has been incubated prior to injection, than when both are injected simultaneously.

Zinsser discusses the possibility of this poison being derived from the medium in which the bacteria are grown. He rules out from consideration peptone and histamin, but indicates that in his opinion, the matter is not as yet finally settled.

Zinsser and his associates have found that many different bacteria will induce the formation of heat unstable toxic substances in young cultures. The formation of this substance is roughly proportioned to the growth energy. The toxic products are essentially similar in the symptoms they elicit in rabbits, and in their harmlessness for guinea pigs. They differ in some essential properties from the classical endotoxins of the same organisms, and they seem distinct from most of the usual toxic substances produced by the cleavage of culture ingredi-

[10] Besredka and Ströbel: Soc. de Biol., 1911, lxiii, 691. Centralbl. f. Bakt., Abt. 1. Ref., 1921, liii, No. 10.

ents. The difficulty of performing serologic work, he states, is increased by the fact that repeated small doses often lead to marasmus, loss of hair, and eventual death of the animal treated.

Certain results suggest an aggressive action of these poisonous products. Sublethal doses of streptococci contained in supernatant fluid from centrifuged specimens have died in two or three days.

Conditions Necessary for Bacterial Growth.—For the general bacteriologist, the number of bacteria submitted to study is tremendously large. Only a comparatively small number are, however, capable of maintaining their existence in the living tissues, either of plants or animals. Those organisms, whose life history is best carried out, in the presence of inert, nonviable substances, are known as *saprophytes:* those which multiply and grow luxuriantly in living tissues are termed *parasites.* Bacteria which are unable to thrive in living tissues are obligate saprophytes, while, on the other hand, those to whose existence living tissues are necessary as a foodstuff are obligate parasites.

Saprophytic bacteria are unable to thrive in the animal body, partly because they are unable to procure the proper constituents for their metabolic activity and because the temperature is not suitable, but also because there are present in the normal body, substances, whose presence is positively inimical to their growth. As will be noted later in this volume, it is possible for the body cells to acquire the property of producing such antisubstances which render impossible the growth of even obligate parasites. Parasitic bacteria, although they are able, for the most part, to remain viable outside the body, do not thrive, since they depend for their nutriment upon the presence in their immediate vicinity of certain of the intermediate products of cellular metabolism. Parasitic bacteria are, apparently, unable by themselves to bring about the splitting of the more complex molecule, with the liberation of the amino-acids which are necessary for their nutrition.

Bacteria, as a class, are living forms which show a very pronounced daintiness in their choice of foodstuffs. For the growth of different types to take place it is necessary that a sufficient supply of oxygen, water, and of nitrogen, hydrogen, carbon, and other elements such as sulphur, iron, etc., be present in an assimilable form.

Moisture.—All microorganisms, in common with other species of living matter, require for their growth a sufficient supply of water, although for the most part moderate desiccation, unless very prolonged, does not result in death of the cell body.

Oxygen.—The majority of pathogenic microorganisms require free oxygen for their growth, and are known as obligate *aerobes.* Upon the other hand there are a certain number, notably the tetanus bacillus and the bacillus aerogenes capsulatus, which can multiply only in the absence of free oxygen; these are obligate *anaerobes.* Still others are able to adapt themselves to the presence of oxygen and are likewise able to utilize the oxygen present, in combination in the substances upon which they live. Such are called facultative anaerobes or aerobes.

Since the living body tissues normally contain a certain amount of free oxygen, infection by obligate anaerobic microorganisms is unlikely to occur, unless the tissues have been injured, and a certain number of cells have become devitalized as the result of trauma or ischemia. Thus, it is a well-known clinical fact, exemplified in so many tragic cases during the war, that infection by bacillus aerogenes capsulatus (B. welchii), rarely takes place unless there be extensive bruising of the tissues.

The great majority of pyogenic and other pathogenic bacteria are aerobes; the most important anaerobic bacteria are the *Bacillus welchii* just mentioned, the tetanus bacillus, and the various spirochetae—*Treponema pallidum* (Noguchi), *Spirochete pertenuis* (Nichols), and the *Bacillus fusiformis*—the cause of Vincent's angina, noma, and phagedenic ulcer (Tunnicliffe).

Temperature.—The majority of pathogenic bacteria grow best at a temperature which approximates that of the human body, 37° C.,[11] 98½° F., although between the range of from 20°-42° C. most bacteria demonstrate fairly active growth.

The highest temperature at which active growth is exhibited is called the *maximum* temperature of growth, the lowest is known as the *minimum*. The degree of heat at which cultivation is most luxuriant represents the *optimum temperature* for that particular species.

Factors Determining Death of Bacteria.—We have briefly noted the factors which tend to develop the most active growth on the part of pathogenic bacteria. It has been shown that, although it is possible to induce most bacteria to grow outside the body, a combination of favorable circumstances as regards food, oxygen supply, moisture and temperature, is, under normal conditions, very infrequently met with. With the exception, therefore, of certain of the anaerobic bacteria, notably the *Bacillus tetani* and *Bacillus aerogenes capsulatus*, which perhaps proliferate in the excreta of the horse, and other animals, we find that very few pathogenic microorganisms multiply outside the animal body. In other words, it is necessary for the propagation of the great majority of pathogenic bacteria that they find entrance into the tissue of man, or some other animal body.

Although, as just stated, the parasitic nature of pathogenic bacteria necessitates their entrance into animal bodies, in order that they may multiply, such an environment, is by no means essential for the maintenance of their vitality, or capacity for growth.

Oxygen.—Although the presence of oxygen, in the immediate environment of bacteria, in such a state that the anabolic processes of the bacterium may be able to utilize it in the physiology of the cell, is essential to the continued vitality of aerobic bacteria, nascent oxygen, such as is liberated from

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[11]Although the subject has been hitherto insufficiently investigated there is much which suggests that the determining factor in infections of the superficial tissues of the body is the fact that the causative agents grow best at a temperature below that of the internal organs.

H_2O_2 or represented by ozone, is capable of rapidly destroying most bacteria, if they are in a vegetative state. In fact the activity of many germicides is due to the liberation of nascent oxygen, and the consequent oxidizing property of the substance.

Desiccation.—Exposure of bacteria to desiccation, or drying, is followed in the course of a comparatively short time (1-24 hours) by death through the removal of water from the cell body. As might be expected, the encapsulated bacteria and those surrounded by the fatty, or waxy covering, which characterize the tubercle and leprosy bacilli, prove more resistant to this factor, than do others not so protected. It is for this reason that there is such grave danger of "house infection" occurring with tuberculosis.

Heat and Cold.—Although a certain degree of warmth is necessary in order that growth of bacteria may take place, and above and below the maximum and minimum limits, respectively, proliferation does not occur, the vitality of the microorganism is by no means destroyed, unless the degree of heat be considerably increased. It is found, also, that even extreme conditions of cold, such as repeated or continuous freezing injure bacteria only after long periods.

Most vegetative forms are unable to resist a temperature of from 56-60° C. for ten minutes, if the microorganism be exposed to such heat in the presence of moisture. Certain bacteria, notably tubercle and leprosy bacilli, are not killed (in ten minutes) by a temperature of 70° C. Moist heat at a temperature of 100° C. such as is obtained by boiling or the steam sterilizer without pressure (Arnold), kills all vegetative bacteria almost instantly.

The destruction of bacteria by means of heat, is apparently brought about by coagulation of the albumin of the bacterial cell, and, since this takes place more readily in the presence of moisture, it is found that higher temperatures are necessary if dry heat be employed. For purposes of sterilization temperatures of 120° C. or more, for several minutes, must be employed; if spores be present, and they must always be as-

sumed to be present in dressings, and similar materials, for the sterilization of which dry heat is commonly employed, an exposure to a temperature of 140° C. for twenty minutes is necessary.

For the complete sterilization of spore-bearing organisms either dry heat may be used, or steam under pressure, in an autoclave, may be employed. For this purpose, a temperature of 125° for a few minutes is sufficient. Red heat, as in a flame, results in instant death of all bacteria and is a method to be strongly recommended whenever possible.

Factors Which Determine Relative Virulence of Bacteria

It is a well-known fact that different strains of the same type of bacterium, or the same strain when exposed to a different cultural environment, possess varying powers of infectivity, and relatively, greater or less, capacity for producing morbid and lethal changes in their host. The sum total of these properties of pathogenicity, constitute virulence. Virulence refers, therefore, to the relative offensive and defensive properties of bacteria and depends upon: (1) the adaptability of the microorganism as regards foodstuffs, oxygen content, etc., found in the tissues of the host, (2) its capacity of resisting the action of proteolytic substances in the body fluids, both natural and immune, of the host, and (3) its capacity for injuring both constitutionally and locally the tissues of the infected individual.

All nonpathogenic bacteria or saprophytes are such, presumably because they are unable to adapt themselves to conditions which maintain in the animal tissues, particularly insofar as these conditions refer to temperature, oxygen content, and foodstuffs, or because the normal proteolytic animal ferments are potent to destroy the bacterial cell bodies.

Highly pathogenic bacteria are such, primarily, because they are able to grow and multiply in the tissues owing, in the first place, to the fact that they find conditions favorable as regards foodstuffs, oxygen, temperature, etc., and secondly, to the fact

that they protect themselves against the activity of the body fluids and cells by various means.

Capsule Formation.—One of the chief protective properties of bacteria is an elaboration, or special modification, of the cell membrane, or enveloping capsule. As a means of protecting itself against the proteotropic antisubstances in the serum, not only is the usefulness of the protective envelope most obvious, but also most efficacious.

Certain pathogenic bacteria, when grown in the animal body, or in media rich in uncoagulated albumins, such as serum, hydrocele fluid or milk, surround themselves with a definite capsule or mucoid material. When such bacteria are cultivated upon ordinary media they lose, largely or altogether, the property of producing a capsule, and can be induced to return to their capsule-producing stage only by means of reinoculation, into suitable animals, or, less readily, by transplantation into albuminous media. The bacteria which belong to this group are, the Bacillus pneumoniae of Friedländer, the Bacillus of rhinoscleroma, Bacillus anthracis, Bacillus aerogenes capsulatus, the plague bacillus, Bacillus cholerae gallinarum and pneumococcus, certain of the streptococci, Micrococcus tetragenus, and certain yeasts, e.g., blastomyces.

It can be readily proved that, coincident with the loss of the capsule forming property of such a microorganism as the pneumococcus, its power of infecting mice, is lessened and that phagocytosis of the bacterial cell *in vitro* is more readily accomplished.

Horiuchi[12] described an experiment in which he employed a micrococcus tetragenus having a dense capsule which resisted phagocytosis almost entirely and killed guinea pigs in a dose of 100 organisms. When this microorganism was grown for a number of days, on rather dry agar, it lost its capsule-forming power permanently, became subject to phagocytosis, and did not affect guinea pigs, even in doses of one thousand million. Rosenow has made similar observations upon bacteria of the streptococcus-pneumococcus group.

[12]Horiuchi quoted from Simon: Infection and Immunity.

In a large number of microorganisms, although true capsule formation has not been demonstrated, an analogous alteration in their physical structure occurs. Under certain conditions of growth in suitable animal tissues, a thickening of the ectoplasm or cell membrane occurs, so that the individual members appear to be much larger than when grown upon simple media. This thickening is more marked in the more virulently pathogenic strains. "This is true especially of the colon and typhoid bacilli and the streptococcus, and leads to appearances which often contrast strongly with the tiny attenuated forms which one is accustomed to see, in old cultures, on the ordinary media" (Simon).

It is readily understood, upon the basis of the protective property of the capsule, why animal inoculation may frequently lead to an exaltation in virulence of a bacterial strain. Still another group of bacteria are normally, and even under saprophytic conditions of life, enclosed by a protective membrane, which renders them almost impervious to the action of harmful influences in the body fluids of the host. These are the microorganisms of the acid-fast group, i.e., the tubercle and leprosy bacilli. These bacilli are surrounded by a fatty or waxy envelope. That the entrance of bacteria of this type is not followed by a clinically fulminant and acute infection, is due, not so much to the fact that antisubstances are produced, as that their rate of growth is extremely slow and that they produce no toxins. Other members of this group, e.g., the timothy hay bacillus of Mueller, do not produce spontaneous morbid changes in human beings, not so much because the antisubstances of the serum destroy them, but because the environment, particularly as regards temperature, is unfavorable.

Although there is apparently no doubt but that the capacity of a bacterium for producing about itself a more or less impermeable enveloping capsule or membrane determines its potential pathogenicity or infectivity, this property is not the only one which is altered during the process of increasing virulence. Thus, in certain instances, it is found that the passage through one animal not only does not increase its

virulence for other species, but may even have the opposite effect. In this way, it may be shown that the virulence of the bacillus of chicken cholera is increased for the fowl by passage through chickens, but is not affected in its action upon the guinea pig.

Obviously, therefore, other devices are at the command of the bacterium for increasing its resistance against the deleterious influences present in the host. The exact nature of these changes is but very imperfectly understood, but they are doubtless due to the acquisition of altered biologic affinities, whereby the organism is able to employ different materials as foodstuffs, and possibly to neutralize the action of injurious substances. That such should be the case seems very natural, and that direct proof of this hypothesis should be difficult is equally easily understood.

It must be realized that the process of exaltation of virulence of bacteria by animal passage, or other attenuation by means of cultivation under more or less unfavorable conditions, does not, necessarily, depend upon the acquisition of new, or the marked development of latent properties, in all the individual members of the strain under observation, but represents, in all likelihood, a survival of the fittest. Thus it may be assumed, that in every collection of bacteria of the same species, certain members will be found in whom the capacity for growth in living tissues, the ability to form capsules, and other properties which may help them to maintain their vitality in the tissues, is more highly developed than among their fellows; many of the latter may, in turn, adapt themselves more readily to the saprophytic life.

When cultures containing representatives of both these groups, are inoculated into animals, only those individuals in whom the defensive attributes are highly developed survive. It is their descendants that persist and multiply, and may subsequently be recovered from the tissues. Thus by means of a process of elimination of the less fit, combined with, in all probability, an educative development, there is produced a strain of increased virulence. If, on the other hand, subcul-

tures to a less favorable medium be made, only the less parasitic or more saprophytic members survive and the strain is said to have been attenuated. In this way is explained the fact that certain bacteria, such as the tetragen of Horiuchi referred to above, may, upon cultivation upon simple media lose, for all time, their pathogenic capacity; also the observation, first made by Pasteur, that although it is possible to exalt the virulence of a species up to a certain point, there is ultimately reached a point beyond which no amount of animal inoculation will increase the virulence of a strain—the *virus fixe* of Pasteur.

The majority of pathogenic bacteria, although by means of variations in cultural environment their virulence may be reduced, maintain, albeit in a more or less latent state, their capacity for protective property formation. Bacteria of this class may well be termed, as has been done by Simon, potential parasites. It is on account of this persistence of potential pathogenicity that such great care must be exercised in the employment of "nonvirulent" strains in artificial immunization, more particularly of human individuals.

Whether bacteria possess the capacity for secreting substances which directly neutralize the antibacterial substances in the body fluids, as is believed to be the case by Bail, Welch and others, and to which the name aggressins has been given, is decidedly problematic. That certain well-defined properties of bacteria, in addition to morphologic changes, capsules, fatty envelopes, etc., are of value to the bacterium in protecting it from the activity of inimical serum bodies, or in so affecting the tissues in which they grow that the defensive function of the tissue cells is, more or less, paralyzed, is easily possible of proof. Certain of these potentialities are liable to modification and are thus of importance in determining the virulence of different strains of the same bacterial species, or the same strain under altered conditions of environment.

Many of the bacteria which are commonly classified as pathogens possess but few, if any, qualities of the true parasite since they are almost incapable of multiplication within living

tissues, but demand in their immediate neighborhood necrosis or devitalized tissue which acts as a pabulum for their growth. Chief among these are the tetanus bacillus and the Bacillus aerogenes capsulatus. In addition there is a large group, which, though possessing much more marked infectiveness, require, for their active proliferation the presence in their immediate vicinity of devitalized tissue. Many bacteria possess the capacity of secreting toxins, which are capable of devitalizing tissues in their immediate environment, and thus supply for themselves the requisite supply of pabulum.

The human tissues quickly destroy the cell body of the diphtheria bacillus as is evidenced by the fact that the administration of antitoxic serum, although this has little bacteriolytic power, not only mitigates the symptoms, but leads to the eradication of the bacilli from the focus of accumulation. The pyogenic cocci, streptococci and staphylococci, manufacture a toxic substance—leucocidin—which inactivates and destroys the most potent means of defense at the disposal of the host.

Other bacteria, notably, the Bacillus welchii (Bacillus aerogenes capsulatus) liberate, from the broken-down tissue consequent upon their growth therein, substances (gas accumulation and toxins) which are potent to destroy the tissue in their vicinity *en masse*, and to lead to thrombosis of the vessels. Thus, it is noted, that although traumatic necrosis is almost invariably necessary, in order that this bacterium may be able to gain a foothold in the tissues, although a generalized infection occurs, only *sub finen vitae,* the extension of the local disease process is often extremely rapid.

The capacity for toxin production varies greatly under different cultural conditions and is greatest, as has been demonstrated by Kendall, when organisms are grown in a medium rich in uncoagulated albumins and practically disappears, or becomes latent, if an excess of carbohydrate foodstuffs be supplied.

Danysz suggests a very interesting point of view with reference to the exaltation of virulence on the part of bacteria.

This suggestion is similar to that brought forward by Welch a number of years ago. The essential feature of this hypothesis is that the animal body may be considered as an antigen for the infecting microorganism and that this antigen provokes the formation of an antibody in exactly the same way, and by the same mechanism, as the fixing substance of the bacterium is antigenic for the animal body.

He refers to experiments in which paratyphoid bacilli, which are virulent for field mice but not for the common rat, may be induced to demonstrate pathogenicity for the latter animal. He says, "In the last analysis the substance of the bacteria acquires a specific affinity for the rat substance and it is thanks to this acquired affinity that the bacteria, or more exactly its own specific substance, can fix and digest the rat substance and render it assimilable."

A series of studies by Savtchenko show that although anthrax bacilli are very rapidly destroyed in rat serum, if a small quantity of serum be added to a large quantity of ordinary bouillon, growth of the anthrax bacillus can be obtained. By continuing passages through mixtures containing relatively larger quantities of serum, we finally obtain a fairly luxurious culture in pure rat serum. These properties of serum resistance are maintained for a period after transplantation into the ordinary bouillon. Again, if the bacteria are removed from the culture by filtration and a small amount of the filtrate is added to rat serum, and this mixture is added to broth, a growth of even a nonserum resistant strain of the bacillus is obtained. It is thus seen that the excess of fixing substance which the bacterium has learned to produce is thrown off into medium in which it is growing, and that this substance can neutralize *in vitro* the bactericidal properties of the serum and render it more assimilable for a nonserum resistant race. It is interesting in this connection to note that contrary to supposition a culture of anthrax—virulent for rats—does not become virulent when rendered serum-resistant (Danysz).

Factors Influencing Infection

The normal epithelial covered surface of the body is sufficiently resistant to prevent the entrance of the great majority of microorganisms into the tissues. On the other hand the numerous glandular structures, sebaceous and sudoriferous, and mucous, are often the site of bacterial growth. From such foci invasion of the parenteral tissues by bacteria frequently occurs. Thus develop acne and folliculitis. A like protective power on the part of the epithelium of the lining of cavities and glandular structures is by no means so well developed, especially if there be an adjacent collection of lymphatic tissue. Numerous experiments have proved (Hess), for instance, that bacteria pass through the mucosa of the intestine and enter the lymphatic and blood streams, in the absence of any discernible lesion of the epithelium; there is no doubt that thus occur many infections with the B. tuberculosis, as well as typhoid fever.

The most susceptible portion of the body to infection is, apparently, the lymphatic tissue of the nasopharynx, especially the tonsils, and the *appendix vermiformis*. Here, as the result chiefly of the deep indentations (crypts) or follicles and the macerating effect of the moisture which covers the surface, as well as the changes brought about by bacterial growth, the protective property of the epithelium is overcome and microorganisms find entrance into the lymphatic tissue. Thus in addition to the well-recognized and readily appreciated affections such as acute tonsillitis (streptococcus and pneumococcus infections), diphtheria and Vincent's angina, entrance to the tissues by this route is probably exemplified in many instances of scarlet fever and other acute infections, e.g., cerebrospinal meningitis, acute anterior poliomyelitis, influenza, leprosy and tuberculosis. Since many of the microorganisms producing these pathologic conditions are normally present in the mouth, their parenteral invasion is known as autoinfection. Similarly, bacteria which gain entrance through the appendix may be carried to the duodenum and gall bladder.

In order that infection may take place, it is necessary that not only must pathogenic bacteria invade the tissues, but in addition they must be capable of multiplication. Actual infection, therefore, depends upon the focal presence of viable parasitic bacteria in tissues in which reactive processes are not stimulated, or being stimulated prove inadequate. It must be recognized that there occurs normally, in such situations as the tonsils, a constant adaptation of the cells to changes induced by the entrance of bacteria into the tissues. So long as this adaptation is adequate and sufficient protective activity is demonstrated by the tissues, no infection occurs. If, on the other hand, there be local loss of resistance, owing to trauma, vascular constriction (cold and exposure), or constitutional depletion of reactive forces, bacteria which gain entrance may obtain a foothold even though under normal conditions of the host their proliferation would be impossible.

It is evident that the bactericidal properties of the serum and tissues can be directed against bacterial cells only insofar as they can be brought in contact. Unfortunately, however, it is not necessary for the multiplication of bacteria that they be in localities supplied by actively resistant powers. It is thus possible for pathogenic bacteria to remain viable and to grow upon the surface of the body, and of the nasopharynx, or within such tubes as the alimentary and genitourinary tracts, without being subjected to adequate resistance on the part of the tissues. Such bacteria are a constant source of danger to the individual host, in the event of his resistance being lowered from any local or constitutional cause, and, also, to those with whom he comes in contact, through the medium of droplet infection, water contamination, etc. Individuals who harbor pathogenic bacteria, although not themselves suffering from disease in any noticeable way, are known technically as "carriers."

CHAPTER III

IMMUNITY AND IMMUNIZATION

Introduction

Purpose of Immunological Study.—"The fundamental task of immunology is to investigate the reaction of the living organism to the invasion of foreign material. In the higher organisms this reaction, so far as we know at present, takes two forms; it manifests itself both in the phagocytic activity of certain special cells and in the production of certain substances known as antibodies" (Weil[1]).

As has been already indicated, an appreciation of the chemical nature and biologic habits of bacteria and other pathogenic microorganisms is essential if the physician or surgeon is to understand the rôle played by them in the production of disease. The complementary science to the bacteriology of disease is that which deals with the means at the disposal of the body, through the exhibition of which the latter guards its tissues against infection, and overcomes and eliminates invading organisms once these have become established. In addition, the experimental investigations of more recent years have proved that the tissues may employ the same means to protect themselves against the harmful effects of certain nonviable foreign colloidal substances, more particularly proteins.

The science which attempts to identify such processes, to analyze their nature and manner of action, and to apply for therapeutic and diagnostic purposes the data thus obtained, is known as *immunology*. The state of the animal body, by virtue of which it is protected against injurious agencies, is termed *immunity*. The process as the result of which the body becomes immune is termed *immunization*.

[1]Weil: Jour. Immunol., 1916-17, ii, 399.

For many years the science of immunology dealt exclusively with the means at the disposal of the body for guarding its tissues against infection and for the elimination of invading microorganisms. With the discovery of the phenomenon of anaphylaxis, it became evident that, to a considerable extent, the fact that bacteria and protozoa are viable proliferating microorganisms is, from the immunologist's point of view, incidental.

Gradually it has become more and more apparent that the science of immunology deals with the reaction of the tissues to the parenteral presence of protein substances, particulate or in solution. The viewpoint of the immunologist at the present time, therefore, embraces not only the reaction of the tissues (either by the elaboration of soluble intra- or extra-cellular substances—antibodies—or by cellular and vascular inflammatory reactions) to invasion or infection by living microorganisms, but also such phenomena as serum sickness, asthma, eczema, pollenosis (hay fever), and perhaps such conditions as traumatic fever, and many degenerative lesions. More recent observations suggest that traumatic shock and acute intestinal obstruction are closely related to certain immunologic phenomena.

It is at once apparent that, under our definition of immunity, we include those means at the disposal of the body, by virtue of which it is protected against:—(1) the harmful effects of nonviable foreign substances, and—(2) infection by living proliferating microorganisms.

The science of immunology seeks to determine:

(a) The nature of the properties at the disposal of the body by virtue of which it guards itself against infection, and, conversely, the nature of the state of the body as the result of which the individual is susceptible to infection.

(b) The manner in which these properties are exalted in the natural cure of infection.

(c) Artificial means whereby the natural development of protective properties may be augmented, whether for the pur-

pose of protection against infection, or in order that bacteria already established in the tissues may be eradicated.

(d) Methods for the identification of specific properties of the body tissues, more particularly of the serum, in the diagnosis of present or past infection, or for the purposes of prognosis.

As the result of the study of the above questions, the attention of the immunologist has been directed to:

(e) The effects upon the host of the parenteral entry into its tissues of heterologous proteins and their derivatives, e.g., horse serum, plant pollen, etc.

(f) The alterations which take place in the constitution of such heterologous proteins following their introduction into the tissues.

(g) Attempts to determine the nature and manner of action of the deleterious agents, which are responsible for clinical manifestations of tissue irritation and injury, in such obscure phenomena as traumatic shock, acute (and chronic) intestinal obstruction, and certain types of tissue degeneration.

The first two subdivisions of the subject apply to the phenomena which accompany infection and the subsequent course of infectious disease, and, as such, form the basis of all rational and scientific study of such affections. The second two subdivisions attempt to apply such knowledge in a practical manner in the diagnosis of disease and the treatment of infected individuals. Sections E and F are the result of the natural progress of the science in the direction of an appreciation of basic principles. Section G serves to indicate an entirely new function of the immunologist, which has been assumed in recent years. Previously employing known irritant substances, he has attempted to determine their effects upon the tissues. He has now undertaken to attempt to prove the nature of unknown agencies, the activity of which has hitherto been recognized only through their effects upon the tissues.

Historical.—Several outstanding phenomena of disease are appreciated by all observers and are being constantly exem-

plified in clinical experience. For instance, it is a matter of daily experience to note that the majority of infections run a definite and, usually, self-limited clinical course, and that, following a state during which the infecting microorganisms gradually increase in number, there ensues some change in the relationship of host and invader, as the result of which the latter is eventually exterminated. Furthermore, the occurrence of one infection and the consequent reaction thereto is followed, in the majority of instances, by a protection from, or at least an altered reaction to, subsequent invasion by the same bacterium.

The recognition of such facts as the natural cure of disease, and the relative protection of the individual against subsequent infection which is conferred by previous attacks of infectious disease, is not recent. For many centuries it has been the custom among certain of the Oriental peoples purposely to infect young and healthy individuals, in order that they might be rendered insusceptible to such diseases during after years. Such a method was at one time (1718) introduced into England from the East by Lady Montague, as a means of combating the spread of smallpox. The inherent danger of such a method both to the individual and to those about him, was sufficient to prevent this method of immunization from becoming generally adopted.

The principle of such immunization methods was, however, before long put to a safe and practical use by Edward Jenner (1796) in his employment of the virus from cases of cowpox for the immunization of human beings. It is of interest to note that Jenner established his procedure after a study of the subject by the method of observation followed by experiment. From the time of Jenner almost a century passed without further notable investigation into the principles of immunology. It remained for Pasteur (1880), who started the work as a chemist, to open up the field for the tremendous amount of work which has been undertaken in all parts of the world during the past four decades.

Pasteur engaged himself in the investigation of the broad

principles of acquired immunity. He was successful in proving that, by means of the inoculation of microorganisms, attenuated by means of aging (chicken cholera), cultivation at high temperatures (anthrax), and by desiccation (rabies virus), it was possible to induce changes in the tissues of animals as the result of which the latter are able to withstand inoculation by virulent strains.

The next step in the development of the science was due to the recognition of the fact that it is possible to transfer, by means of the serum of immunized animals, protective substances to other normal animals. The names of those who did pioneer work in this direction include Salmon and Theobald Smith (hog cholera), Brieger and Kitasato (tetanus), and Roux and Yersin (diphtheria).

In Germany, Paul Ehrlich commenced work upon the phenomena of immunization. His experiments led him to formulate the first series of hypotheses which attempted to indicate the underlying principles. Inasmuch as Ehrlich's terminology has impressed itself well-nigh indelibly upon the literature of immunology, and since part at least of his hypothesis has been very generally accepted, a short review of his opinions, insofar as these refer to the mode of development of antibodies, is included.

Ehrlich postulated in this regard that the living cell, whether of animal or vegetable origin, is enabled to anchor and assimilate nutritive foodstuffs only by virtue of its possessing an affinity for certain specific molecules present in its environment. To such affinities the name *receptor* was given. Not only are such receptors capable of combining with and anchoring useful nutritive substances, but also of fixing noxious or harmful bodies, such as bacterial toxins. According to this hypothesis, it is only such tissue cells as have specific receptors for specific toxins that are susceptible to injury therefrom. Ehrlich's next step was to assume that the power of producing such receptors is practically unlimited, and that, once the cell has been stimulated to produce them, it continues to do so for a very considerable length of time. Stimulation is accom-

plished by the simple effect of exhausting those receptors normally present. Furthermore, Ehrlich assumed that, when increased production of receptors is stimulated, large numbers of the latter are discharged out of the cell and find their way into the general circulation. Receptors thus circulating have the same affinity as those attached to the cells, and, since it is believed that the junction of receptor and toxic principle results in detoxication of the latter, the circulating receptors protect the fixed receptors from the injurious effects of injected irritants.

For a number of years it appeared as if each new record of experiment rendered an understanding of the subject more complex, and suggested an ever-increasing number of factors. The last few years, however, have given evidence of a tendency towards simplification and simplicity. The theories of Ehrlich have been productive of an enormous amount of work, and have directly and indirectly stimulated most valuable experiment and observation. The attempt, however, of observers to harmonize their actual findings with the demands of Ehrlich's hypotheses have occasionally led to an apparently unnecessarily complicated and, at times, false conception of the fundamental principles of immunologic reactions.

The multiplication of terms to describe substances identified in the serum of immune persons and animals, and the employment of diagrammatic representation which has suggested the conception of synthesis rather than cleavage as the predominating principle in immunity reactions, have made it unnecessarily difficult for the student and practicing physician to gain a proper grasp of the subject.

In 1883 Metchnikoff commenced the publication of a series of articles which had for their chief purpose a more adequate appreciation of the importance, in the elimination of tissue irritants, of phagocytic activities on the part of blood and tissue cells. Metchnikoff, who had studied and attained eminence as a zoologist, was prepared to find in the migratory cells of the human and higher animal tissues an exhibition of those properties which characterize the life activity of the unicel-

lulae. With such a preparation for the study of reactions of the tissues to irritants, it is easy to understand why Metchnikoff believed the phagocytic properties of the cells to be almost exclusively important. Since Ehrlich's postulates indicated that the immune state was due almost exclusively to the presence in the body fluids of soluble circulating substances (antibodies), and since Metchnikoff claimed greater importance for direct cell activities, there arose two schools of workers known as exponents of the humoral and cellular[2] theories, respectively. That both cellular phagocytosis and antibody production are necessary for the practical prevention of, or cure of, infectious diseases, is obvious, so that, to a great extent, the division of workers into so-called humoralists and cellularists has largely ceased. It seems to have been proved that, provided the body cells, more particularly the polymorphonuclear leucocytes, can be induced to take part in protective reactions, their functioning is more effective and economical of body effort than is the simple exhibition of antibody activity.

In 1905, and the following year, experiments were described by Richet (France), Rosenau and Anderson (United States), Otto (Germany), and von Pirquet (Austria), which dealt with a phenomenon which, though previously noted,[3] had not attracted the attention it deserved, and its importance had not been appreciated. The essential feature of this phenomenon, to which the name of anaphylaxis was given by Richet,[4] is the fact that normal animal tissues are so altered by the parenteral[5] introduction of a foreign protein that, although the majority of such proteins are harmless (in moderate doses) to normal animals, the injection of even minute doses of the same protein after the lapse of twelve or more days is followed

[2]It should be noted that the humoral and cellular theories here considered are in no way related to the modern humoral and cellular theories regarding the site of the anaphylactic reaction which are discussed at length in a later chapter.

[3]Theobald Smith, Flexner, etc.

[4]Richet's first observations were published in 1902.

[5]Parenteral by routes other than the digestive tract.

by the immediate manifestation of symptoms of severe (commonly fatal) intoxication.

The discovery of the phenomena of anaphylaxis and allergy has had such a vital influence upon the science of immunology that a proper conception of their nature is essential, if we are to be able to follow the recent, and future, advances in the knowledge of immunity processes. At the present time no subject is interesting immunologists more than that which has to do with the relationship of anaphylaxis or hypersensitiveness to immunity. That these phenomena must be related to one another has been accepted by practically all observers, and much time and effort have been expended and many experiments performed by numerous investigators in the hope of proving this relationship.

For many years immunologic experiments were carried out almost exclusively *in vitro*. Bacteria and sera were placed together in test tubes, and on the results observed were based inferences as to the processes which take place in the living body.

Gradually more and more experiments have been undertaken upon living animals. Von Pirquet pointed out the necessity for such form of investigation and devised the method of introducing the irritant into the superficial layers of the skin and noting the nature, and extent, of the reaction exhibited. In association with Schick he studied inoculation with the causative agents of vaccinia, smallpox, measles and recurrent fever, and the effect of injections of serum, streptococcus suspensions, tuberculin and mallein. Upon the results of these experiments they based their hypothesis that substances, of the nature of antibodies, react with the foreign materials and that the products of such reaction function as poisons to the tissues, and consequently provoke hyperemia and cellular accumulation. The period of incubation is the time necessary for the formation of antibodies.

Other important experiments of recent years bearing upon our understanding of immunity principles, are those which have studied the physiology of cell digestion and utilization

of protein foodstuffs, and the appreciation of the fact that digestion may, under certain circumstances, be accomplished parenterally (i.e., within the tissues as opposed to enteral or intestinal digestion). With the development of our knowledge of biologic chemistry, along the lines indicated above, the names of Fischer and Abderhalden are associated.

Elementary Facts Regarding Immunity

Immunity: A Relative Term.—Immunity is usually but a relative term. When the statement is made that a certain animal or person is immune, it does not necessarily follow that it is impossible to induce infection of such individuals, but that, under normal conditions of health, they are not injured by doses of microorganisms which are potent to produce grave injury, or even death, in other individuals. Thus it is found that, although the hen is not susceptible to infection by even large doses of pneumococci, so long as the hen be otherwise in normal health, if the hen be exposed to the injurious action of prolonged cooling in cold water, infection may be produced.

Similar phenomena indicating the effect of exhaustion as the result of loss of sleep, insufficient food and overwork, as well as the deleterious action of chilling of the body, are common in clinical experience. How frequently is the alcoholic debauch, with its accompanying improper feeding and exposure to cold and wet, followed by the onset of a pneumococcal or streptococcal infection, even though these two microorganisms are among those against which the adult members of our urban communities are immune to a considerable degree.

Natural Immunity.—If a mouse and a hen be inoculated with cultures of the same virulent pneumococcus, it will be noted that, whereas the mouse rapidly dies with symptoms of grave intoxication, the hen is unharmed by the inoculation. Such experiments prove that certain animals are not susceptible to the injurious action of bacteria which are pathogenic for members of another species. Such animals are said to possess *natural* immunity to infection. Examples of such

natural immunity are not uncommon in man, as for instance against hog cholera, chicken cholera, coccidiosis, leucoplasmodia, and other blood parasites.

Although such phenomena are commonly grouped under the heading of natural immunity, it is probable that the insusceptibility of the individual to infection is due, not so much to the presence of "immune bodies" in the body fluids, as to inability of the infecting agent to proliferate under the conditions which exist in the tissues of the host.

Acquired Immunity: Active and Passive.—Clinical experience proves that an attack of measles, or typhoid fever, usually protects the individual from subsequent infection with the same virus or bacillus. Such an insusceptibility to, or protection from, infection is acquired through the active stimulation of the body's resisting properties, and is therefore called *active acquired immunity.* A similar active immunization may be induced by means of the injection of nonvirulent or dead microorganisms, as in the prophylactic immunization against rabies by Pasteur's method, or the employment of dead typhoid bacilli after the manner of Wright. To such methods of immunization the qualifying term *artificial* may well be applied.

By means of the employment of the blood fluid (serum) obtained from animals, or human beings, which have been themselves actively immunized, either as the result of the natural cure of disease, or through artificial inoculation, normal individuals may be rendered immune for a longer or shorter length of time (seven to twenty days). The resistance to infectious agents obtained in this way is known as *passive* or *transferred immunity.*

CHAPTER IV

GENERAL PRINCIPLES OF ACQUIRED IMMUNITY AS EXEMPLIFIED BY TOXIN-ANTITOXIN REACTION

Soluble antibodies, which may be demonstrated in the serum of immune animals, may be divided into two fundamental groups, namely, (1) simple antitoxin formation, and (2) those responsible for the more complicated phenomena of proteolysis. In addition, there is exhibited by the tissues the property of development of tolerance to the irritant products of the proteolytic reaction. Since the means whereby toxins are inactivated is a simple one, and is readily studied experimentally, and since many of the basic immunologic principles are exemplified by this phenomenon, it is the first to receive our attention, and is employed to illustrate certain broad principles which characterize all immunity reactions.

In Chapter II, it was noted that certain bacteria, notably *B. diphtheriae, B. tetani,* and *B. botulinus,* produce soluble poisonous substances which are known as toxins. Similar toxins may also be derived from a number of vegetable substances, e.g., abrin and ricin, and are contained in the poison of snake venom. One of the qualifying characteristics of the poisons, which are called true toxins, is that they give rise to the production of specific antisubstances known as *antitoxins,* when injected in sublethal doses into suitable animals.

Toxins bring about the death of certain cells in consequence of their ability to combine with these cells. This fact is of basic importance, especially since it indicates the reason specific diseases are characterized by the involvement of different organs or groups of cells. By means of the employment of known chemical poisons, e.g., strychnine, atropine, morphine, phenol, arsenic, etc., it is possible to injure various tissues in a specific manner and, thus, to imitate the pathologic states encountered in clinical experience.

Again, the symptomatology of serum sickness, which is caused by an absolutely nontoxic and innocuous foreign protein, such as horse serum, can in many respects not be differentiated from that of an acute infectious disease. This fact indicates the great importance of the processes that are involved in the reaction of the cell to foreign protein, and is essential to the comprehension of some of the most common phenomena of clinical medicine, as well as of cellular physiology in a general sense.

Briefly, the theory underlying such facts is that certain cells are composed in part of specific unsatisfied or unstable molecules, which have an affinity for certain substances, which may be essential to the life of the cell and therefore beneficent, as for instance the amino-acids and other foodstuffs, or may be noxious and lead to degenerative phenomena or even death in the cells and thus to incomplete or deranged function on the part of the organ to which they belong. It is to these unsatisfied molecules that Ehrlich has given the name *receptor.*

At the first stage in the elimination of foreign protein, the cells must first anchor it; such fixation of the antigen stimulates the production of new receptors (antibodies), and finally the foreign protein disappears from the body. In the absence of antibodies, it may remain in the body indefinitely. The absence of affinities on the part of any of the tissue cells appears to govern the resistance of white mice and rats to diphtheria toxin. "Thus we may conclude that antibody formation or the immune response is the necessary condition for the elimination of certain proteins from the body, and that the prime requisite for antibody formation is the anchoring of the foreign protein by the cells" (Weil).

There are instances of foreign proteins which the cells of the body do not appropriate out of the circulation. This has been shown to be true especially of certain toxins. Thus, green lizards, the marsh turtle, and some other animals, are not susceptible to intoxication by tetanospasmin. It is characteristic of these animals that the toxins remain in their circulation for a long period of time, even months. Thus

Metchnikoff found that a lizard, kept at a temperature of 20° C., and injected with an amount of toxin sufficient to kill 500 mice, at the end of two months still retained in its blood such an amount of the poison that 0.1 c.c. caused fatal tetanus in a mouse.

Such animals are insusceptible to the effects of the poison for the reason that their cells do not anchor it. They retain the foreign substance in their blood for an indefinite period, simply because their excretory organs are not adapted to eliminate it. "But there is still a third, and most important, corollary of this inability of the cells to fix the poison, namely, the complete absence of antibodies from their blood at any time whatever subsequent to the injection of the poison" (Weil).[6]

Only such cells as have the property of combining with heterologous protein molecules are injured by them. Similarly, only such cells as normally possess affinities (receptors or normal antibodies) are concerned in the elaboration of free antibodies.

Toxin-Antitoxin Immunity.—If we inject a guinea pig with less than the minimal lethal dose[7] of *bacillus tetani* or its toxins, allow seven to ten days to elapse, and repeat the inoculation, employing one and one-half times the M.L.D. of material, the guinea pig may, perhaps, show symptoms of tetanus, but is not destroyed by the injection.

Similarly, we may inject a guinea pig, intraperitoneally or otherwise, at intervals of one week or ten days, with six gradually increasing doses of tetanus toxin, commencing with one-half or one-quarter the M.L.D., and eventually employing four or five times that amount. In such an experiment it is found that, ten days after the last dose, it is possible to inject the animal with several times the M.L.D. without provoking fatal intoxication.

We have thus proven that the repeated introduction of

[6]Weil: Jr. of Imm., Vol. ii, 1916-17, p. 408.

[7]The smallest dose of a toxin which is sufficient to bring about the death of the animal within a certain definite time (twenty-four hours) is termed the minimal lethal dose (M.L.D.).

toxins into the body of a guinea pig is followed by the development, on the part of the animal, of an insusceptibility to its injurious action. Such an insusceptibility is known as acquired, in contradistinction to natural immunity. As will be presently seen, acquired immunity develops not only against toxins but against protein substances and against bacterial cell bodies.

It is evident that there may be two explanations of the development of this insusceptibility of the animal to the harmful effects of a specific toxin. Either the receptors, owing to whose presence the cells absorb the toxic substance and thus bring about their own injury, have been used up, or exhausted, in absorbing, or neutralizing, the original sublethal dose; or substances may have been produced which render the toxin inert and incapable of injuriously affecting the cells. It is possible to prove that, usually at least, this form of immunity is due to the development of an increased number of receptors or antibodies, which possess an affinity for the specific toxin, whose presence in the tissues has stimulated their production, and, also, that these bodies are present in the body fluids. In consequence they neutralize, or detoxicate, the injected, or focally developed, toxin, and so protect the tissue cells.

Passive Immunization.—If the guinea pig, employed in the last experiment, be bled, and the serum obtained be injected intraperitoneally into a normal guinea pig, the latter animal will be protected against the effects of a subsequent injection of toxin. This may be proved by injecting the animal which received the dose of serum from the immune[8] pig with one and one-half times the M.L.D. of tetanus toxins. We find that the animal may sicken, but does not die.

By such an experiment we demonstrate that it is possible to transfer protection against the action of tetanus toxin to a normal animal, by means of the introduction into its tissues of the serum of an immune animal. Such immunity as is thus

[8]An animal which is protected as the result of the presence of specific antibodies present in its body fluid is said to be immune.

produced is termed "passive" or "transferred" immunity, and is clinically exemplified by the employment of antidiphtheritic, or antitetanic, serum for prophylactic, or therapeutic, purposes.

The protection of the immune animal against the pathogenic action of bacterial toxin is evidently due to the presence in its serum of substances capable of rendering inactive the toxic material. This can be further demonstrated by an experiment such as the following, in which the toxin and the serum, which contains the inactivating substance (antibody), are mixed *in vitro*.

If three cubic centimeters of the serum from an immune animal be added to twice the M.L.D. of diphtheria toxin in a test tube and the whole be placed at a temperature of 37° C. for one hour, the subsequent injection of the mixture into a normal animal is followed by no manifestations of toxemia.

Toxin and antitoxin mixtures, if suitably graded quantitatively, are thus shown to be capable of neutralizing one another in the test tube. Furthermore, it can be proved that the introduction parenterally into the animal body of toxin-antitoxin mixtures is followed by an immune body production on the part of the tissues of the animal inoculated.[9]

A further fact is demonstrated by these experiments, namely, that, as the result of stimulation of the antibody-producing centers through the medium of a specific irritant, e.g., tetanus toxin, there is induced an increased production of immune bodies. That the production of antitoxins is of a specific nature is proved by the fact that, if snake venom or ricin be employed in the immunization of an animal, antitoxins will be produced which possess the property of neutralizing the poison, for instance cobra venom, by the injection of which the animal had been immunized, but which are not potent to protect the animal from poisoning by diphtheria toxin.

This characteristic of specificity of immune substances is common to all types of antibodies; it must be noted, however,

[9]This fact is made use of clinically in that it is now recommended that children that prove themselves by the Schick reaction to be susceptible to diphtheria toxin be immunized in this way.

that against irritants, which are closely related to one another biologically, there are produced to a greater or less extent common (group) antibodies. Thus antitoxic serum against cobra venom is found to be active, though less potent, against the venoms of other snakes.

So far as has been discovered, the antitoxins present in immune animals are identical in nature with those present in normal individuals, and vary only in their marked increase in concentration. The same rule applies, moreover, to all antibodies about to be described.

More important is the observation that, following the cessation of the stimulus, the elaboration of antibodies continues, so that eventually, at the end of one or two weeks, there accumulates in the serum of the animal a very considerable excess of antibody over the probable needs of the individual.

If an animal which has received an injection of less than one M.L.D. of toxin be inoculated, or its serum tested, *in vitro* or by transferred immunity experiments, within two or three days after the first injection with toxins, it is found that no protective property of the serum can be demonstrated. After the third day, the presence of antibodies may be proved. An increase in antibody content continues gradually, and reaches its maximum from twelve to twenty days after injection. According to Knorr[10] one diphtheria toxin unit injected into the horse may lead to the production of 100,000 antitoxic units.

From the time of greatest production of antibodies, there occurs a gradual decrease in the amount of recognizable antibody. The length of time during which immune bodies remain in the tissues, in excess of the normal, is apparently by no means a constant one; the sera of many animals apparently remain potent up to 10 years or longer, whereas others appear to lose their antibody content after a lapse of but a few weeks.

From this brief study of antitoxic bodies several outstanding facts are recognized, which, it may be remarked, are characteristic of other immune bodies as well:

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[10]Knorr: München. med. Wchnschr., 1898, p. 321, 362.

1. The animal body is capable of producing, if properly stimulated, antitoxic substances which are potent to destroy or neutralize certain types of poisons (toxins), especially those produced by bacterial activity.

2. The proper stimulation to elicit such a response consists in the parenteral introduction, in sublethal amounts, of the specific toxin against which antitoxins are desired.

3. Once such antitoxin production has been induced, it continues, so that eventually the serum of an animal may possess many million antitoxic units as compared with the normal.

4. The permanence of the antitoxic substance in the serum of immunized animals is a more or less variable one, in many instances persisting for a term of years.

In the foregoing experiments we have dealt with the production of antibodies (antitoxins) against certain nonviable poisonous substances known as toxins. The interaction of these substances is a perfectly simple one and can be carried out equally well in the test tube as in the animal body, nor is it necessary that the serum used be fresh.

Certain of the physical characteristics of antitoxins are indicated as follows:

(1) They are soluble substances.

(2) They withstand desiccation.

(3) When in the dry state, the antitoxic properties of sera are maintained for years unaltered.

(4) The antitoxin substances are contained almost exclusively in the globulin portion of the serum.

(5) The antitoxic property of serum is not altered by trypsin digestion.

The changes in the constitution of the blood serum as the result of immune body development must be extremely subtle in character. They cannot be noted by physical or chemical means, such as alteration in the index of refraction, specific gravity, or reaction.

That the combination of toxin and antitoxin, as of other immune bodies, is not a chemical binding but rather a physical

absorption (colloidal reaction) seems probable from the fact that it is possible to inactivate completely the antitoxic property of a serum by treatment with a quantity of toxin considerably less than the amount which might be neutralized by the same quantity of antitoxin. This is shown by the following type of experiment. Suppose the potency of an antitoxic serum be such that 2 c.c. are sufficient to prevent death following the introduction of two M.L.D. of toxin; it is found that, if 2 c.c. of this serum be treated with a quantity of toxin equal to one-quarter the above amount the subsequent addition of one M.L.D. will not be neutralized. In consequence death of the animal follows injection of the mixture. To this characteristic of antibodies the term absorption (Bordet) has been applied.

Bordet has likened the relation of toxin and antitoxin, or the affinity of antitoxin for toxin, to that which occurs when blotting paper is placed in a dye such as methylene blue. For instance, it is found that, if a dyestuff be prepared of such a strength that 100 pieces of paper will be tinted a given blue, if all are introduced at once, this does not take place if the papers are added seriatim. The first pieces, in this event, take up from the concentrated solution more than their share of the dyestuff, so that by the time the last pieces are added, practically no tinctorial property will remain to the solution.

CHAPTER V

ANAPHYLAXIS—HYPERSENSITIVENESS

Introduction

In 1839 Magendie noted that rabbits which had been injected with egg albumen died after a repetition of the injection. A typical anaphylactic experiment was performed and described in 1894 by Flexner. "Animals," he wrote, "that had withstood one dose of dog serum would succumb to a second dose after a lapse of some days or weeks, even when this dose was sublethal for a control animal."

In 1896 Theobald Smith observed that occasionally guinea pigs appeared to lose their natural resistance to certain foreign substances, notably serum, when reinjected with small quantities of a like material.

It was not, however, until 1906 that the results of investigations were published almost simultaneously by Rosenau and Anderson, by Richet, and by Otto, which served to establish the essential principles which underlie such protein hypersensitiveness. These observers proved that, after the lapse of a period of two weeks following the parenteral introduction of numerous substances of a protein nature, which are harmless to untreated animals, subsequent injection of the same material is followed by striking manifestations of intoxication or death.

It appeared at first, that this phenomenon was to invalidate many of the observations which had previously been made upon immune processes, since the essential characteristic of the immunity reactions, which had been studied, consisted in an insusceptibility to, or resistance against, infection of the tissues. More extensive experiments and observations have, however, harmonized many of the apparent discrepancies between the two reactions, and gradually the importance of the phenomenon

of anaphylaxis or hypersensitiveness, and its relationship to immunity has been recognized.

Certain facts concerning the phenomenon of anaphylaxis are of the utmost importance if the practicing physician, or surgeon, is to be able to analyze the clinical manifestations of disease, and to intelligently guide, and assist, the tissues to prevent infection, or to eliminate invading microorganisms.

The important features of the phenomenon of hypersensitiveness, are that certain tissue cells, stimulated by the presence in the tissues of foreign proteins, elaborate an antibody, through the activity of which subsequent introduction into the tissues of the same protein, is followed by symptoms of marked irritation, or intoxication. Although the manifestations of tissue irritation differ in different animals, it is a fact that in each species of animal the same clinical phenomena and autopsy findings are uniformly exhibited, no matter what the source or nature of the protein.[1]

Gradually it has been more and more appreciated, so that now it is universally recognized that anaphylaxis is an immunologic phenomenon. This being the case, it must be presumed, that the process serves a useful purpose in the physiology of the body.

The observations of Rosenau and Anderson permitted them, as early as 1908, to summarize the fundamental characteristics of the anaphylactic reaction in the following way. Horse serum, which is harmless in moderate doses to normal guinea pigs, if injected into these animals, in even minute doses, renders them after a definite interval of time, hypersensitive to subsequent injections of the same material. The interval necessary for the development of the hypersensitive state with doses of 1 to 2 c.c. is about ten days. The reaction is specific: injections of horse serum sensitize to horse serum only. The sensitive condition is transmissible from mother to offspring. The young of sensitized mothers are susceptible to anaphylactic shock at the first injection of horse serum.

The anaphylactic state can be induced by injections of

[1]This subject is dealt with in greater detail in the Chapter on Nature of the Antigen.

various animal and vegetable proteins, and also by extracts of various bacteria. The bacteria used by Rosenau and Anderson in their experiments included colon, anthrax, typhoid, and tubercle bacilli.

Richet, some years previously, had given the name "anaphylaxis," in contradistinction to "prophylaxis"—or protection from—to any evidence of hypersensitiveness exhibited by an animal. Rosenau and Anderson[2] accepted the term "anaphylaxis" to designate the phenomenon investigated by them. Since this time the reaction has been commonly known by this name.

In 1905 von Pirquet and Schick[3] published the first of their classic studies upon serum disease and vaccinia[4] reactions. They recommended the use of the term "allergie" (allos—altered, ergia—reaction), to indicate the phenomenon studied by them, which it will be seen is apparently identical in principle with that studied by Rosenau and Anderson. Since, for the most part, the phenomena noted by these observers consisted of visible vascular and cellular reactions, the author has reserved the term allergy to indicate the focal morphologic reaction which occurs when hypersensitive animals, or men, are injected into the soft tissues of the body with an antigenic protein.

Definitions

In order that the subsequent experiments and discussions may be rendered more comprehensible, the terms employed by the author are defined. This is particularly necessary since

[2]Rosenau and Anderson: U. S. Pub. Health & M. H. S. Hyg. Lab. Bull., 29, 1906; 30, 1906; 36, 1907; 45, 1908. Jour. Med. Res., 1906, xv, 1907, xvi; also Jour. Infect. Dis., 1907, iv; 1908, v, quoted by Zinsser).

[3]Von Pirquet and Schick: Die Serum Krankheit, Deuticke, Leipzig, 1905. Also Münch. med. Wchnschr., 1906, liii, 67.

[4]In 1798 Jenner in his "Inquiry into the Causes and Effects of Variolae Vaccinae" described the phenomena which characterize the allergic reaction as noted by von Pirquet. He wrote: "It is remarkable that variolous matter, when the system is disposed to reject it, should excite inflammation on the part to which it is applied more speedily than when it produces the smallpox. Indeed it becomes almost a criterion by which we can determine whether the infection will be received or not. It seems as though a change which endures through life had been produced in the action or disposition to action in the vessels of the skin; and it is remarkable, too, that whether this change has been effected by the smallpox or the cowpox, the disposition to sudden cuticular change is the same on the application of variolous matter."

the concepts designated by the same term differ somewhat when applied by different writers.

Anaphylaxis[5] is a phenomenon in animals which have received parenterally[6] small doses of[7] heterologous proteins (albuminous substances) as a result of which they become abnormally sensitive to the subsequent introduction of the same antigen. This abnormal sensitiveness is manifested by the onset of marked symptoms of tissue irritation following the injection of foreign protein, in amounts which are harmless to the normal animal.

The animal subject to anaphylaxis is said to be *sensitized,* or *hypersensitive;* sensitization is induced by means of a *primary* or sensitizing parenteral injection.

The fulminant or explosive anaphylactic reaction noted in animals, particularly guinea pigs, is known as *anaphylactic shock,* or *immediate anaphylaxis* (Auer and Lewis), and occurs when the sensitized animal is injected after a definite period of time, known as the *incubation period,* with a second dose of the same protein. The protein which is injected, in order to prove the existence of the anaphylactic state, is known as the *massive, exciting* or *toxic* dose.

Delayed anaphylaxis signifies the late development, that is, one or more hours after injection, of symptoms which may or may not lead to death of the animal.

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[5]In Wells' opinion the following criteria must be met if the phenomenon following parenteral injection is to be considered as anaphylaxis:

1. The observed toxicity of the injected material must depend upon the sensitization of the animal; i. e., the substance must not produce similar symptoms in nonsensitized animals.
2. The symptoms produced must be those characteristic of anaphylactic intoxication as observed in the usual reaction with typical soluble proteins, being, therefore, the same for all antigens with the same test animal, but differing characteristically with each species of animal.
3. It should be possible to demonstrate passive sensitization with the serum of sensitized animals.
4. It should be possible to demonstrate typical reactions in the virgin guinea pig uterus strip.
5. It should be possible to demonstrate amelioration or prevention of the bronchial spasm in guinea pigs by proper use of atropin and epinephrin.
6. The possibility that the observed symptoms are caused by capillary thrombosis or embolism must be excluded.
7. After recovery from anaphylactic shock there should be exhibited a condition of desensitization under proper conditions.

[6]Parenteral, i. e., by routes other than the digestive tract. In practice the expression indicates introduction of a substance into the tissues, as in intravenous, subcutaneous, intraperitoneal, or intrathecal injections.

[7]Foreign substances derived from sources other than the animal's own tissues.

Allergy[8] (von Pirquet and Schick), as the name implies, is an altered reaction (inflammatory in nature) in the hypersensitive animal which is noted at the site of injection, following the focal introduction of antigen.

Anaphylactin[9] or *allergin* is the substance (antibody) assumed to be present in the tissues of sensitized animals which acting upon, or reacting with, its specific protein antigen is responsible for the manifestation of the anaphylactic phenomenon. It is to this substance that I have applied the term Immune Body of the First Order (1st Order Body).

The term *anaphylatoxin* (Friedberger), or split product, is applied to the toxic substance which is believed by many observers to be developed in the tissues as the result of the interaction of antigen (foreign protein), first order body (anaphylactin), and alexin (complement). It is worthy of note that the terms "*endotoxin*" (Pfeiffer) and "*apotoxin*" (Richet), signify in all probability the identical substance.

Anaphylactic or hypersensitive animals which have received sublethal doses of the specific protein so that they do not react to immediate further injections with the same protein are said to be *desensitized.*

In the literature regarding anaphylaxis much confusion has arisen regarding the use of the terms *antianaphylactic, desensitization,* and the condition referred to as *refractory,* and immunity. The author believes that it is wise to dispense with the use of the terms "refractory" in speaking of the anaphylactic reaction. As a general rule the animal is said to be in a *refractory condition,* when in consequence of the administration of a sublethal dose of protein antigen the hypersensitive animal has been desensitized.

Desensitization is due to exhaustion of the antibodies present in the tissues, which through their reaction with the protein

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[8]Wells employs the term allergy to cover all those manifestations of altered reactivity which cannot be included under the heading anaphylaxis. Anaphylaxis is looked upon by him as forming one particular form of allergy.

[9]Southard and Gay, it must be noted, have employed the term anaphylactin to designate the remnant of foreign protein circulating in the blood following a sublethal shock. Southard and Gay: Jour. Med. Research, 1907, xvi; 1908, xviii; 1908, xix.

antigen are responsible for the manifestations of the symptoms of tissue irritation which characterize anaphylactic shock.

The term *''antianaphylaxis''* has been used to indicate both the phenomenon of desensitization, and also the *tolerant* state induced in animals in consequence of repeated parenteral injections of a protein antigen.

If the term ''antianaphylaxis'' is to be employed at all, it should, in my opinion, be reserved to designate the latter condition (tolerance).

In general the author believes that an understanding of the subject is more easily acquired if the terms (a) ''anaphylaxis'' or ''hypersensitiveness,'' (b) ''desensitization'' and (c) ''tolerance'' be employed to designate, respectively, the following:

(a) *Hypersensitiveness,* the state of the animal tissues in consequence of which it is hypersensitive to the introduction of small doses of specific protein antigen.

(b) *Desensitization,* a condition in which, in consequence of a sublethal injection of the specific protein antigen which exhausts the bodies responsible for the anaphylactic reaction, the animal, for the time being, is comparable to the normal animal; and is not susceptible to anaphylactic shock.

(c) *Tolerance,* that state of the tissues, acquired in consequence of repeated sublethal injections of protein antigen, in which the animal, even after a lapse of two or more weeks, is tolerant or immune to the introduction of doses of the protein antigen which suffice to induce fatal anaphylactic shock in hypersensitive animals.

CHAPTER VI

FUNDAMENTAL PHENOMENA CHARACTERIZING HYPERSENSITIVENESS, DESENSITIZATION AND TOLERANCE IN THE GUINEA PIG

In this introductory chapter an attempt is made to briefly review the established characteristics of the phenomena of anaphylaxis and tolerance, and, also, to indicate an hypothesis, regarding their relationship to one another and to immunity processes in general, which, in my opinion, is sufficiently well supported by experimental data and clinical observations to deserve adoption as a working theory.

When the animal tissues are injected with a foreign protein, at first there is no apparent effect. After an incubation period (eight to fourteen days) the further introduction of the same protein, (or the presence of a residual quantity of material from a previous introduction) is followed by manifestations of irritation or intoxication of the tissues.

If the route of administration be suitable, and the size of the intoxicating dose sufficient, explosive symptoms, respiratory or circulatory in character, or both, ensue. Such fulminant symptoms occur more particularly if the route of administration be intravenous. If the protein be injected into the soft tissues, or into serous cavities, focal inflammatory reactions occur. With suitable dosage febrile reactions of varying intensity may be provoked.

Striking and interesting phenomena are exhibited when half-grown guinea pigs are subjected to repetition of injections of the same antigen at varying periods. Guinea Pig A may be given within five days of the first injection from 0.2 to 2.0 c.c. of sheep serum without the manifestation of untoward symptoms.

Guinea Pig B fourteen days after a preliminary (sensitizing)

injection of 0.5 c.c. of sheep serum receives an intravenous injection of 0.5 c.c. of sheep serum. Within a period of one or two minutes the animal exhibits symptoms of grave irritation and dies within from three to eight minutes in a state of asphyxia.

The above constitutes the typical anaphylactic experiment in the guinea pig, and is known as anaphylactic shock.

Guinea Pig C is injected upon the same day as B with an intraperitoneal dose of 0.2 c.c. of sheep serum. There follows no immediate onset of symptoms, but within a period of one-half to one hour the animal is found to be suffering from malaise; the animal's hair stands on end and the pig huddles itself in a corner; it may shiver, and is obviously unhappy.

The temperature at first drops slightly, and then shows an elevation above the normal; a transient leucopenia occurs, which is followed by leucocytosis. A scant purulent exudate may be recovered from the peritoneal cavity. The animal completely recovers within twenty-four hours or less.

We have thus discovered that if an animal which is susceptible to anaphylactic shock be given a small dose of protein, by a route which permits of but slow absorption into the circulating blood stream, a simple febrile reaction accompanied by malaise may be elicited.

Upon the following day Guinea Pig C is injected in a manner (intravenous), and with an amount of serum, similar to Guinea Pig B. No symptoms of intoxication are exhibited. This and other experiments prove that this animal has been desensitized by the injection of a sublethal dose of the specific antigen; while in this state the animal is said to be refractory.

Guinea Pig D receives, upon the fourth day after the original injection of 0.2 c.c., an intraperitoneal injection of 2.0 c.c. of sheep serum, and, thereafter, a like amount on three or more successive occasions at intervals of five or six days.

After a lapse of from twelve to sixty days after the last injection, a dose, similar to that introduced into Guinea Pig B, is injected intravenously. No reaction takes place.

This experiment proves that repeated sublethal doses of an antigenic protein render the recipient animal tolerant (or

immune) to doses of the antigen, which are fatal to animals rendered hypersensitive by the injection of but one moderate dose of the antigen.

Another animal prepared in the same way as Guinea Pig D is injected intravenously with a larger dose, 3.0 c.c. of sheep serum. Typical anaphylactic shock with death supervenes.

Thus it is demonstrated that an animal which receives repeated doses of protein, although tolerant to doses of antigen which are fatal to hypersensitive animals, remains hypersensitive to this protein, but that larger exciting injections must be employed in order to provoke anaphylactic shock.

Another pig, Guinea Pig E, fourteen days after a sensitizing injection of 0.2 c.c. of sheep serum, is bled to death and its serum recovered. The serum thus obtained is injected into a normal pig with the result that the latter becomes highly hypersensitive. In order that such a transference of the anaphylactic state may be brought about, it is necessary to inject from two-thirds to the total amount of serum recovered.

Similarly, it is found that if the serum of a tolerant animal treated in the manner of Guinea Pig D be injected into a normal pig, the latter becomes passively hypersensitive. It is found, moreover, that but a very small amount of serum, e.g., 0.1 c.c., is necessary.

The paradoxical phenomena are thus noted, that the serum of an animal whose hypersensitiveness is easily demonstrated, contains but a small number of units of the anaphylactic antibody, whereas the serum of a tolerant animal contains a very large number of units of the same antisubstance.

Obviously the animal D is not desensitized as was C, but has developed a state which may be described as tolerance or immunity to the protein antigen. At the same time it is noted that the more tolerant (immune) an animal may be, the greater is its potential hypersensitiveness.

In a later chapter it is shown that it is possible to transfer passive tolerance to a normal animal by the employment of large doses of the serum from the repeatedly injected animal and also to confer passive tolerance upon an actively sensitized

animal such as Guinea Pig B. How, then, can the relationship of these two conditions of hypersensitiveness and tolerance (immunity) be explained?

In answer to this question the author suggests the following hypothesis. The parenteral introduction of heterologous proteins into the animal body is followed by the elaboration, by certain tissue cells, of specific antibodies, which so react with the protein antigen that an irritant[1] substance is developed. In consequence of the presence in the tissues of this anaphylactic antibody, the animal is hypersensitive to the reinjection of the specific protein as the result of whose primary injection the production of the antibody was stimulated. Subsequent parenteral introduction of the same antigen in sublethal doses results in the stimulation of the production of a second order of antibody[2] which is potent so to alter the irritant which arises from the reaction between the antigen and the first order body as to render it innocuous to the body cells.

For purposes of description I have called the anaphylactic antibody the "immune body of the first order." The substance capable of so altering the product of the reaction between antigen and first order antibody that it no longer acts as an irritant is termed the "immune body of the second order."

The immune bodies of the first order, which are responsible for the exhibition of the hypersensitive state, conform to the characteristics of other antibodies, such as antitoxins, amboceptors, agglutinins, and precipitins.

(a) They are produced as a result of stimulation.
(b) They are specific for the antigen employed.
(c) They are produced in excess of the immediate requirements of the organism.

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[1]This irritant substance may be identical with the poisonous split product of Vaughan, or the "anaphylatoxin" of Friedberger.

[2]Although it remains to be definitely proved that the toxic split protein prepared by Vaughan is the same as the product of the antigen first order antibody reaction, there is much to indicate their identity. If such be the case, it has been proved that it is possible to induce the production of the antibody of the second order without the elaboration of those of the first order. Vaughan has performed experiments in which he shows that repeated injection of the poison prepared according to his method is followed by a state (tolerance) as the result of which the animal is able to "bear three or four times the minimum lethal dose," and that, furthermore, such an animal is not sensitive to the whole protein from which the poison was recovered; nor is it possible to sensitize the animal with the split product alone.

(d) They are present in the body fluids, as proved by *in vivo* and *in vitro* experiments.
(e) They may be transferred passively from hypersensitive to normal animals.
(f) They are relatively permanent.

By the repeated introduction parenterally into the tissues of protein antigen, the animal becomes "immune" or tolerant to further injections. The second order antibodies, which are responsible for tolerance, do not conform to the characteristics of the other antibodies. They are apparently less specific than antitoxins or first order antibodies; they are not produced in great excess; they do not persist as long as the first order bodies.

The relationship of the first and second order antibodies to one another is as follows: The anaphylactic or hypersensitive first order body always appears first. The first order body is always present in excess of the amount of second order body, and always persists longer. Tolerant animals are, therefore, always potentially hypersensitive. This is shown by allergic reactions and passive anaphylaxis as well as by means of direct experiments. The tolerant animal, also, ultimately becomes anaphylactic. The second order or tolerant antibodies may be exhausted by the introduction of a suitable dose of the protein, and the animal remains hypersensitive.

Although the author does not personally believe that the objections to the hypothesis of toxic protein split products (anaphylatoxin), arising out of the reaction between anaphylactin and antigen, as brought forward by Weil, Schultz, Zinsser and others, are sufficiently direct to invalidate the hypothesis, he is anxious that the argument regarding the nature, source, and site of production (intra or extracellular) of the toxic substance, which is responsible for the manifestations of tissue irritation which characterize the phenomenon of anaphylaxis, should not obscure the important facts, that, whereas a single small parenteral introduction of protein renders the recipient animal anaphylactic, repeated doses of the same protein are followed by the development of a state of tolerance.

CHAPTER VII

MORE DETAILED DESCRIPTION OF THE PHENOMENA OF ANAPHYLAXIS IN THE GUINEA PIG AND OTHER ANIMALS

If a guinea pig weighing 250 grams be injected, intravenously, or intracardially, with 4 c.c. of normal horse or goat serum, the animal, although it may exhibit symptoms of irritation for a short time, does not die and rapidly returns to normal.

A second guinea pig of a similar weight to the one employed for the above experiment, is injected by a parenteral route with a small quantity, 0.1 c.c. or even less, of heterologous serum, and after the lapse of a period of two weeks receives a reinjection of 2 c.c. of the same serum directly into the blood stream; within from thirty to ninety seconds the onset of anaphylactic symptoms is exhibited.

Clinical Phenomena of Shock in the Guinea Pig.—The animal at first appears excited, then commences to twitch, scratches its nose and may cry out; there rapidly follow symptoms of respiratory difficulty. The animal commences to "buck"; within one or two minutes there is evidence of weakness or paralysis of certain muscles, especially of the hind legs. There is, commonly, also, an involuntary discharge of urine and feces. The animal falls upon its side, and suffers from marked expiratory dyspnea. This is accompanied by a gradually increasing cyanosis. Death supervenes in from ninety seconds to six or seven minutes from the time of injection. The heart frequently continues to beat for several minutes longer.

Other characteristic phenomena consist of a very marked fall in blood pressure and drop in temperature. The complement content of the blood is decreased, according to Fukuhara, and others, to from one-half to one-third the normal quantity.

The leucocytes in the peripheral blood are diminished and the coagulation time of the blood is much increased.

Similar acute reactions may be induced in the guinea pig by the intracerebral or postorbital route of injection of the toxic dose.[1]

If, instead of the intravenous route, the injection be made into the peritoneum, the sequence of events is somewhat less striking. Larger doses of protein antigen must be employed and death rarely takes place in a shorter time than five or six minutes. If small doses be employed in this way, the animal, after the exhibition of mild symptoms of tissue irritation, appears to be fairly normal. If the dose be sufficiently large, the pig dies in a comatose condition, from five minutes to two hours after injection. It may be noted that guinea pigs, which recover from the intravenous injection of protein antigen, rapidly return to their normal state of health and rarely succumb to the late onset of symptoms.

Anderson[2] has described the ordinary type of reaction which occurs when the antigen is injected into the peritoneal cavity as follows: "Within five or ten minutes the guinea pig becomes restless and agitated; it runs about the cage and sometimes utters sounds of distress; then there appear manifestations of peripheral irritation and respiratory embarrassment. This is shown by scratching at the mouth, coughing, sneezing, rapid and irregular respiration. An exceedingly characteristic feature of the respiratory involvement is that, at intervals, the animal makes an unusually deep inspiratory effort with the diaphragm, resulting in a marked sinking at the lower end of the sternum. The stage of excitement is soon followed by one of paresis, or in some cases complete paralysis. The animal is unable to stand and, if it attempts to do so, it falls upon its side."

Guinea pigs in a condition of complete paralysis may fully recover, but within a short time convulsions usually begin and

[1]The postorbital route of injection has been employed extensively by Baldwin and Krause and in their hands has proved a most successful and reliable technic. It is used as the method of choice by Besredka.

[2]Anderson: Bull. Johns Hopkins Hosp., 1910, xxxi, 218.

are almost invariably the forerunner of a fatal termination. Occasionally in guinea pigs, which are not very sensitive, the onset of the symptoms, following an intraperitoneal injection, may be delayed thirty or forty minutes, but in only a few instances has Anderson noted the onset of symptoms delayed as long as an hour.

Postmortem Findings in Guinea Pigs Dying in Anaphylactic Shock.—Autopsy upon guinea pigs which die as a result of anaphylactic shock demonstrates, almost invariably, the following characteristic lesions: The whole body is cyanotic, the lungs are distended and collapse but little when the thorax is opened; they are pale, somewhat grayish pink in color, and usually show beneath the pleura numerous irregular ecchymotic patches varying in size from 0.5 to 4.0 millimeters in diameter. Occasionally, also, there is a more extensive hemorrhage, and the pleural cavity is found filled with fluid blood. Subepicardial hemorrhages are also frequently seen as well as, in one case noted by the author, diffuse hemorrhage into the liver substance. The longer the animal survives the injection, the greater is the evidence of venous congestion of the abdominal organs.

In the guinea pig, subcutaneous injection of lethal doses of foreign sera are usually followed by an absence of symptoms for a period of one hour. If death takes place, it occurs, as a rule, at the end of from four to six hours. If the animal survives, recovery is evident between the sixth and the twelfth hour. The symptoms manifested under such circumstances are: an unwillingness on the part of the animal to move about; it lies down, shivers, respiration becomes more and more feeble, and gradually in a fatal case ceases entirely. At autopsy upon animals which have died as the result of this delayed reaction, fatty degeneration of the parenchymatous organs and multiple hemorrhages throughout the body are characteristically found (Longcope, Boughton).

Accompanying nonfatal shock, following intraperitoneal and subcutaneous injection of the protein antigen, there occurs a

febrile reaction and the blood exhibits an increase in the number of circulating leucocytes—leucocytosis.

In the guinea pig sensitiveness may, as has been stated, be induced by way of the intravenous, intraperitoneal or subcutaneous routes, and also as in man, by the digestive and respiratory tracts. No other laboratory animal has shown itself to be so readily affected as the guinea pig, although, as stated above, the rabbit, dog and man, as well as goats, sheep, horses, mice, chickens and pigeons, are susceptible. In other words, the reaction constitutes a phenomenon of general biologic significance.

Anaphylaxis in the Rabbit.—Rabbits are less susceptible to the fulminant rapidly fatal shock which has been just described in the case of the guinea pig. Relatively larger doses of serum must be employed in order to bring about a fatal termination in these animals. Death is, moreover, gradual and is not characterized by dypsnea but by gradual collapse as evidenced by fall in blood pressure and lowering of body temperature.

Subcutaneous injections of foreign serum into hypersensitive animals are followed by the development of an urticarial eruption similar to that which characterizes serum sickness in man. Under suitable conditions of hypersensitiveness on the part of the animal and size of protein dose, local edema, necrosis, and abscess formation may occur at the site of injection—Arthus' phenomenon. The maximum reaction is exhibited at the end of twenty-four hours.

Arthus[3] also studied anaphylactic shock in the rabbit; the following is taken from his description of the reaction.

A rabbit which has been previously treated with antigen three times, received 2 c.c. of horse serum in the auricular vein. After one minute the animal began to sneeze, became anxious and restless. It lay on the abdomen, the respiration became frequent, loose passages of the bowels occurred, then the rabbit rolled onto its side, threw its head back and kicked its extremities. It then became quiet and ceased breathing.

[3]Arthus: Comp. Rend. de la Soc. Biol., lv, 817; loc. cit., lv, 1478. Reunion biol., Marseille, June, 1903.

After a short pause a few respirations occurred, and the animal finally died about four minutes after the injection.

Anaphylaxis in the Dog.—The dog is more difficult to sensitize than is the guinea pig. Repeated parenteral injections of the antigenic protein are usually necessary. In the dog the symptoms of anaphylaxis are, as a rule, less violent than those which take place in the guinea pig, and death occurs less frequently. Following intravenous injection there is evidenced great restlessness, the animals frequently scream, and pronounced evidence of weakness rapidly supervenes. In those cases which succumb the animal falls over on its side, and may remain motionless for hours. Defecation and urination take place, apparently involuntarily. Dyspnea is not marked but there is a *rapid and marked fall in blood pressure.*

This fall usually begins about forty seconds after the beginning of the intravenous injection of the antigen. The pressure usually reaches a minimum of about 25 mm. of mercury by the end of ninety seconds. In shocks of moderate severity, the pressure remains at this low level for about twenty minutes, and then gradually increases, reaching normal in from one to two hours, depending on the severity of the reaction. With highly sensitized dogs, injected with relatively large doses of the specific protein, little or no recovery takes place, the pressure remaining at a low level until the death of the animal, which usually occurs in about forty minutes. (Manwaring.[4])

This phenomenon has been found to be due to a peripheral action on the part of the poison upon the splanchnics. (Biedl and Krause.[5])

In those animals which recover, once the blood pressure begins to rise, the other symptoms rapidly disappear. In most cases blood is discharged from the intestines and there may be developed a condition of hemorrhagic inflammation in both the large and small gut, which Schittenhelm and Weichdardt[6] have

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[4]Manwaring, Chilcote, and Hosepian: Jour. Am. Med. Assn., Feb. 3, 1923, lxxx, 303.

[5]Biedl and Krause: Wien. Klin. Wchnschr., 1910, No. 11; also "Kraus u. Levaditi Handbuch," Erganzungsband 1.

[6]Schittenhelm and Weichdardt: Deutsch. med. Wchnschr., 1911. 19.

termed "*enteritis anaphylactia.*" Leukopenia is constantly present, due chiefly to a loss of mononuclears. There is, too, a marked lengthening of the coagulation time of the blood.

In the dog, the onset of symptoms, even though the intravenous route of administration be employed, is usually delayed, although, as shown by Weil,[7] this is not necessarily the case. Weil prepared dogs by two injections with 5 c.c. of horse serum. After an interval of two or three weeks an intravenous injection of 20 c.c. of the antigenic serum was employed as the toxic dose. "The symptoms following upon this second injection are characteristic and almost constant. The dog immediately vomits or retches, and generally has a number of evacuations of the bowels. Within five minutes it begins to stagger and to drag its hind legs. Following this preliminary state comes a period of severe collapse, which, as a rule, appears within ten minutes of the injection. The animal lies on its side and does not respond to any stimulation. Some animals show at first, either a fine tremor of the muscles of the extremities, or a coarse clonus composed of short excursions. These soon cease, and the animal is practically immobile, except for the respiratory movements. Respiration is either shallow or rapid, or labored, and gives the impression of marked dyspnea. During this stage, which terminates, usually within thirty minutes, with the death of the animal, the other characteristic features of anaphylaxis make their appearance. The blood pressure sinks so low that the carotid pulse can scarcely be detected. If blood is aspirated from the veins it is found to have lost its coagulability to such an extent that it remains fluid for several days."

Postmortem Findings in Dogs Dying of Anaphylactic Shock.—At necropsy, extreme dilatation of the vessels draining the upper abdominal viscera is noted.

The liver, spleen, and kidney are congested, and the veins of the upper intestinal tract are distended. The bowel wall, in rapidly fatal reactions, is deep purple in color.

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[7]Weil: Jour. Immunol., 1916-17, ii, p. 527, 528, 538.

Anaphylaxis in the Cat.—The cat is relatively insusceptible to anaphylactic shock. Anderson and Frost,[8] however, were successful in passively sensitizing guinea pigs with the serum from a cat.

Anaphylaxis in the Goat.—Zinsser[9] has experimented with goats and observed both serum and bacterial anaphylaxis. In these animals the symptoms consist of general trembling, weakness, labored respiration, and involuntary evacuation of urine.

Anaphylaxis in Man

Severe Immediate General Reactions—Anaphylactic Shock

Two cases of anaphylactic shock in man that terminated fatally I have observed, personally, and the notes of one case, hitherto unpublished, have been placed at my disposal.

In those cases in which the splanchnic type of reaction predominates, there occur collapse, pallor, tachycardia, thready pulse, pain in the epigastrium and over the heart, increased intestinal peristalsis, rectal tenesmus, vomiting and blood concentration.

The respiratory type of reaction is characterized by choking sensations, cyanosis, asthmatic dyspnea and arrest of respiration.

The cutaneous phenomena consist of erythema, urticaria and "angioneurotic" edema.

There follows an abstract of a case, report[10] of a healthy man who collapsed following the hypodermic administration of a relatively small dose, 5 c.c. of horse serum, and who died with symptoms during life identical with those which occur in anaphylactic shock in the dog. Necropsy findings were identical with those which are found in the guinea pig.

CASE 1.—Sgt. E., aged thirty, was given 5 c.c. of serum subcutaneously over the right pectoralis major at 11:15 A. M., Sept. 1, 1916. He was in good general condition, the wounds from which he was suffering being trivial.

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[8]Anderson and Frost: Tran. Cong. Am. Phy. and Surg., 1910, p. 431.
[9]Zinsser: Infection and Resistance, p. 370.
[10]Gurd, F. B., and Emrys-Roberts, E.: Fatal Anaphylaxis, Lancet, April 3, 1920, i, 763.

At 1:30 P. M., he commenced to vomit and a bloody diarrhea developed. This gastrointestinal disturbance was accompanied by a moderate degree of collapse. At 5 P. M. his pulse was 104 and his temperature 102.8° F. He complained of moderate headache and thirst; there was slight generalized abdominal tenderness, but no rigidity. He made no complaint of respiratory distress, despite the fact that he was cyanosed and restless. The skin was "goose-fleshed" and covered with a mild erythema.

Until 11 P. M. his condition remained somewhat relieved. No treatment was instituted as his condition was not considered alarming.

At 11 P. M., after a slight vomiting attack, the collapse became very severe. He became pulseless at the radials; the heart beat rose to from 160 to 170 per minute. He became extremely cyanosed, and restlessness increased. During the night, stimulation was pressed to the utmost. Pituitary extract, 1 c.c. to the dose, was given every three hours. One-thirtieth grain of strychnine was given at 11 P. M. and its administration was continued in doses of one-fortieth grain every three hours. He received three pints of saline subcutaneously during the night, and oxygen was given almost continuously up to the time of death. Except for the fact that he became quieter and more comfortable while receiving oxygen, there was no response to stimulation.

At 6 A. M., September 2nd, these notes were made: "Pulse is absent at the wrist, heart rate 180 and feeble. There is a very marked purplish discoloration (cyanotic erythema) of the whole body. This discoloration disappears on pressure and returns very slowly. The extremities are cold. There has been no return of vomiting or diarrhea."

Death ensued at 10:30 A. M., apparently due to cardiac failure secondary to drop in blood pressure. Respiration during the last three hours of life was at the rate of from 48 to 54 per minute.

Necropsy was performed four hours after death. Owing to the presence of pleural adhesions, the lungs were squeezed considerably in the process of removal, nevertheless, they were voluminous and downy, except over the posterior parts which were boggy and dark in color. The lungs were reddish gray and were covered over the whole surface with innumerable subpleural collections of deep purple colored blood. Those patches[11] varied in size from 1.5 to 5 mm.

Beneath the parietal pleura there was a small number of similar hemorrhagic spots.

The cut surface of the posterior parts exuded a considerable amount of bloody, frothy fluid; the anterior portions showed a dilatation of the alveoli and numerous small purplish red spots.

The upper intestinal tract showed slight capillary dilatation.

Insofar as we were able to discover, the patient had not been previously wounded nor had he received, at any other

[11]These hemorrhagic patches were typical of those found in anaphylaxis in the guinea pig.

time, injections of horse serum. That he was an individual presenting a natural hypersensitiveness to horse serum is, therefore, a fair assumption.

Inasmuch as the patient in Case 2 was not under observation by any one medical officer during the period intervening between the time of serum injection and his death, there is perhaps some slight doubt regarding the nature of the collapse that resulted. Correspondence was, however, carried on with those officers who had seen the patient prior to admission to the Casualty Clearing Station, and the note made by the officer in charge of the patient at the main dressing station of the Field Ambulance stated that "while in the field ambulance, he appeared to be suffering slightly from shock, but in other respects was in good condition when he left the main dressing station."

CASE 2.—S. M. was admitted to the Casualty Clearing Station, six hours after being wounded and three hours after receiving serum injection at the field ambulance.

His injury consisted of a perforating wound of the upper third of the left thigh behind the bone. No large vessel was injured; there was no evidence of hemorrhage; the sciatic nerve was intact and there was no important laceration of muscle. Briefly, the wound was of such nature as was considered trivial, infection excluded.

He was admitted at 8:30 A. M. On admission his condition was seen to be extremely grave; he was pale, slaty in color, and mentally stuporous. The mucous membranes were not blanched. The pulse was barely perceptible at the radials; the rate was over 140 per minute. There was no respiratory difficulty, no urticaria; the extremities were cold.

The patient was placed under an incandescent heater with the foot of the bed raised, but no improvement occurred. On the contrary, he appeared to become worse. An operation was, therefore, performed at 2 P. M., as it was feared that despite negative findings he might be suffering from a deep-seated gas gangrene.

Examination of the wound was made under gas and oxygen anesthesia. An intravenous saline solution containing 7.5 per cent glucose, to which was added 8 mm. of epinephrin, was given. Fifteen hundred c.c. was thus injected. During the induction of anesthesia (nitrous oxide and oxygen) collapse of the patient became almost complete. Respiration ceased and the heart sounds became almost inaudible. The rate of flow of the intravenous solution was increased, and artificial respiration was employed. This resulted in return of signs of life. After 500 c.c. of the solution had been injected, there was marked improvement in the patient's con-

dition, and at the time of the completion of the injection of the total quantity of 1,500 c.c. the patient's pulse rate was 86, of good pressure, and his color was good. He became mentally alert and remarked that he had felt "all in," but that he was now fit. The operation itself consisted simply in an incision of the affected part for purposes of examination. Carrel tubes were inserted. The whole procedure occupied but three or four minutes. Examination revealed no evidence whatever of gas gangrene.

He returned to the ward in good condition, "practically normal in appearance," and remained fit for approximately thirty minutes, when he rapidly collapsed, and died at 4:30 P. M. Unfortunately, owing to pressure of work in the operating room, it was not possible to see him during this second period of collapse.

Necropsy was performed thirty minutes after death. There were no positive findings other than enlargement of all the great and smaller veins in the splanchnic area. After removal of the heart, the right thorax filled immediately with fluid blood up to two-thirds of its capacity. Examination of the leg confirmed the absence of gas gangrene.

There is no doubt, in my opinion, that this was a case of fatal anaphylaxis following the subcutaneous administration of a small dose (presumably about 5 c.c.) of antitetanic serum. This belief is based upon the absence of any other cause of shock or infection, and upon the very prompt response to the glucose injection with its epinephrin content. At necropsy, as mentioned above, the only abnormality was the extreme content of blood in the veins and capillaries of the splanchnic area and the absence of clotting one-half hour after death.

Boughton[12] has reported a case (Case 3) of anaphylactic death, occurring in an asthmatic, following an intravenous injection of 1 minim of normal horse serum undiluted.

CASE 3.—"Within two minutes a typical attack of asthma supervened. He was given 10 minims of epinephrin intravenously with definite relief for about ten minutes." Four other similar doses were given, each gave relief for several minutes, but the patient died at forty-five minutes after the injection of serum.

Necropsy was performed within half an hour after death. The face was cyanotic and the lips swollen. "The abdominal cavity showed intense injection of the vessels everywhere, being especially marked in the veins of the stomach, mesentery, gall bladder and appendix. The entire small intestine was bright red and dilated submucous vessels showed distinctly

[12]Boughton, T. H.: Anaphylactic Death in Asthmatics, Jour. Am. Med. Assn., Dec. 27, 1919, lxxiii, 1912.

through the intestinal wall. The parietal peritoneum was markedly injected; no exudate was visible in the peritoneal cavity.

"Both lungs were enormously distended and emphysematous. The left lung showed small areas of hemorrhage at the lateral portion of the lower lobe, about 4 cm. in diameter, with a gelatinous organizing exudate at this point. On section the lungs were found to be dry. Microscopically, the lungs showed passive hyperemia but no edema; there were a few small interstitial hemorrhages." Microscopic examination of the kidneys revealed the most marked changes: "The epithelium of the convoluted tubules was distinctly edematous and there was considerable degeneration and some necrosis; there was intense passive hyperemia. Interstitial hemorrhages were numerous but not extensive."

McCallum[13] reports a case (Case 4) of fatal anaphylaxis following the prophylactic injection of diphtheria antitoxin, subcutaneously.

CASE 4.—The patient was a boy, aged eight, apparently healthy. Following the injection of 2,000 units (amount of serum not stated), death occurred in five minutes. Two minutes after the injection was made, the boy made the complaint that "it had gone to his stomach." He ran out of the house to the privy in the back yard. One minute later he was heard calling, "Daddy, Daddy." His father immediately ran to his assistance and found him completely collapsed and apparently choking. His whole body was deeply cyanosed; he was pulseless. Artificial respiration was employed without avail.

Necropsy was performed. No note regarding the condition of the lungs or splanchnic region is published.

Patrick[14] has reported three cases of anaphylaxis occurring in patients who received intravenous injections of horse serum in the treatment of bacillary dysentery.

The following case well exemplifies nonfatal shock of the respiratory type in man following the subcutaneous introduction of antigen. In addition it proves the possibility of desensitization by means of repeated small subcutaneous injections.

CASE 5.—Lieut. H. was admitted to the Casualty Clearing Station on account of asthmatic attacks, which the patient himself believed to follow proximity to horses. An effort was made to confirm his sensitiveness to horse protein, and to determine the degree of sensitiveness to horse serum.

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[13]McCallum, D.: Fatal Anaphylaxis Following Prophylactic Injection of Diphtheria Antitoxin Subcutaneously, Brit. Med. Jour., Nov. 8, 1919, ii, 596.

[14]Patrick, C. A.: Anaphylactic Shock After Injections of Serum Intravenously, Brit. Med. Jour., July 28, 1917, ii, 114.

At 11:30 A. M. Nov. 12, 1912, 0.25 c.c. of horse serum was injected intradermically into the skin on the outer border of the left upper arm. Fifteen minutes later there was an onset of coryza and an asthmatic attack occurred which increased for about twenty minutes. At its maximum, it was slightly to moderately severe. A marked swelling of the face and ears, more particularly of the eyelids, occurred to such a degree that he was unable to see. The lobes of both ears filled with fluid and hung well down on his neck. At the site of injection there was an edematous enlargement, about the size and shape of a hen's egg, which hung down, having the appearance of a supernumerary breast. This reaction persisted, though gradually subsiding, for from four to six hours.

The effect of this reaction to the injection was more marked than was anticipated and suggests that the dose used (0.25 c.c.) was unnecessarily large for the purpose of confirmation or diagnosis.

The following day, November 13, at 11 A. M. he was injected with 0.15 c.c. of horse serum subcutaneously; no reaction occurred. At 12:30 the same day he received 0.25 c.c. intramuscularly; no reaction either local or general took place. At 2 P. M. of the same day, he received 0.5 c.c. subcutaneously, again without reaction.

The patient was unwilling to remain in the hospital longer, and seemed satisfied to have the cause of the asthma definitely established, hence it was impossible to attempt immunization. The experiment does prove a very great hypersensitiveness to horse protein in a patient who thought himself to be subject to asthma when in the presence of horses, and also the possibility of desensitizing by means of a dose of 0.25 c.c. of horse serum.

The patient was discharged from the hospital with a note to the effect that prophylactic or therapeutic injections of horse serum should not be employed unless such a procedure was preceded by a desensitizing dose.

For the following notes, of a similar case, I am indebted to Major John Fraser, M.C.

CASE 6.—The patient was wounded June 29, 1917. His injury consisted of a small perforating wound of the right upper arm. Five hundred units of antitetanus serum were administered at the field ambulance. Upon admission to the Casualty Clearing Station, three or four hours later, the patient was pulseless, his body was covered by a hyperemic rash, there was a generalized edema of the whole body and he was vomiting frequently. Blood count demonstrated; hemoglobin, 110 per cent; erythrocytes, 8,240,-000, and leucocytes, 25,000.

Calcium lactate was given in doses of 15 grains, three times a day. Improvement in the patient's general condition followed after the third dose.

The following day the patient's condition was satisfactory. Blood count showed: hemoglobin, 82 per cent; erythrocytes, 6,100,000, and leucocytes, 13,000.

An important case of severe anaphylaxis following the administration of horse serum with recovery, is reported by Munro.[15] This case is of interest on account of the adequate description of symptoms, and also on account of the satisfactory result which followed the treatment as instituted by Munro.

CASE 7.—The patient, who had received prophylactic antitetanus serum subcutaneously when wounded, came under Munro's care suffering from erysipelas of the leg. In the treatment of the latter, he was given 30 c.c. of antistreptococcic serum intravenously. The serum was diluted. The extent of dilution is not stated in the article. No effort was made to desensitize the patient. Within one minute after the injection, the patient complained of choking; he grasped his larynx, breathed jerkily and spasmodically. Extreme cyanosis developed and the pulse became flickering. Breathing ceased in full respiration. Treatment was instituted at once; the head of the bed was lowered and artificial respiration was commenced. Epinephrin, 30 minims; and atropin, 1/100 grain were given subcutaneously, and chloroform placed on a mask through which all air which entered the lungs during artificial respiration had to pass.

Within five minutes, the patient's condition improved; normal breathing commenced; the pulse returned at the wrist, and was counted at 150 per minute. The patient later made an excellent recovery.

In this case the reaction was almost exclusively respiratory, in nature, and in this respect should be compared with Case 4 in which the manifestations of splanchnic dilatation predominated.

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[15]Munro, W. T.: A Case of Anaphylaxis, Brit. Med. Jour., Nov. 22, 1919, ii, 668.

CHAPTER VIII

PHYSIOLOGY OF ANAPHYLACTIC SHOCK

The phenomena of immediate anaphylaxis in the guinea pig, described above, are those noted when the animal is allowed to run about. If it be fixed to the holder, other symptoms are more marked. There is noted a sinking of the chest at each inspiration which gradually becomes more marked and, finally, breathing ceases for about one minute. Respiration then commences again, and the mouth is opened at each inspiratory effort. After thirty to ninety seconds, respiration ceases permanently, although the heart continues to beat for a considerable length of time.

Basing their opinions upon the pathologic conditions found at autopsy, especially as regards the lungs, and the clinical symptoms as manifested with the animal attached to the holder, Auer and Lewis[1] were the first to appreciate that asphyxia is the cause of death in anaphylactic shock in the guinea pig. They proved, moreover, by means of experiments, in which animals were kept alive after pithing, that the phenomenon occurred independently of the action of the central nervous system. Biedl and Krause[2] have also proved that the fall of blood pressure, which is the most characteristic phenomenon in dogs, is also due to a peripheral action.

Believing asphyxia in the guinea pig to be due to spasm of the muscles of the bronchioles, Auer and Lewis attempted to inhibit, or prevent, the development of the reaction by means of the hypodermic injection of atropin. In this they were successful. Their results have been repeatedly confirmed. Anderson and Schultz[3] and others have also been successful in modifying the severity of the shock by means of the admin-

[1]Auer and Lewis: Jour. Am. Med. Assn., 1909, liii, 458; also Jour. Exper. Med., 1910, xii, 151.

[2]Biedl and Krause: Wien. klin. Wchnschr., 1909, xxii, 363.

[3]Anderson and Schultz: Proc. Soc. Exp. Biol. and Med., 1909, vii, 32.

istration of chloral hydrate, or of oxygen by the lungs, and by means of adrenalin.

Pearce and Eisenbrey[4] showed by means of experiments in which they employed decapitated animals, which were kept alive by transfusion, that the essential phenomena of anaphylaxis may be brought about independently of the cerebromedullary and spinal centers.

Tissues Affected in Anaphylaxis

The functional disturbances and anatomical results which are exhibited in anaphylaxis differ, as has been seen, with the species of animal employed for experiment, and are dependent upon the route adopted for the introduction of the foreign protein into the body tissues. That the usual absence of the acute symptoms, referable to the lungs in the rabbit, is not due to the nonformation of an antibody, such as that which is responsible for the phenomenon in the guinea pig, is proved by the fact that if the serum from a rabbit which has been sensitized, be injected into a guinea pig, it confers upon the latter a most pronounced hypersensitiveness. It would appear, therefore, that the different manifestations of tissue irritation, in different animals, are due to a varying susceptibility, of their respective tissue cells, to the action of a common irritant responsible for the phenomenon.

Histologic studies of guinea pigs' lungs have shown that the bronchi in this animal have an exceptionally developed musculature. The mucous membrane, moreover, is thick and folded. The finer bronchioles are practically nothing but muscular tubes surrounding a thick folded mucous membrane. Perfusion fluids pass through the distended anaphylactic guinea pig lung without obstruction; spasm of the bronchioles in this animal is not accompanied by spasm of the blood vessels.

In the rabbit lung inflation is of much less importance than in the guinea pig; the symptoms are chiefly circulatory.

[4]Pearce and Eisenbrey: Proc. Soc. Exp. Biol. and Med., 1909, vii, 30.

There is a marked fall in arterial blood pressure; the heart rate is at first slow. Distention of the right side of the heart is found at autopsy. Auer believes that these changes result from a direct effect upon the muscle of the right ventricle. Coca has found that during anaphylactic shock, an increased resistance to the perfusion of fluid through the rabbit's lung occurs. Histologically, the pulmonary arteries of the rabbit present a remarkable degree of muscular development, which is analogous to that of the bronchioles in the guinea pig.

In the dog the liver and splanchnic circulations become tremendously engorged with blood; the systemic blood pressure falls, because of an insufficient supply of circulating blood. Simonds[5] has shown that the walls of the hepatic veins of the dog differ from those of other animals, and that they show greater development of their musculature. He infers that hepatic and splanchnic congestion are the result of spasm of the hepatic veins. It is thus seen that there is reason for tentatively assuming that the characteristic features of acute anaphylactic shock in different animals depend upon difference in the distribution of nonstriated muscle (Wells).

Longcope,[6] Boughton,[7] and others have reported parenchymatous degeneration with secondary fibrosis in the kidneys, hearts, and livers of rabbits and guinea pigs which have been subjected to repeated sublethal injections during the hypersensitive stage. The focal inflammatory reactions, which are considered in this volume under the heading of allergy, are obviously due to the presence of an irritant but not primarily to reactions on the part of unstriped muscle.

The investigations of Manwaring and Crowe,[8] show that three types of anaphylactic or anaphylactoid reactions occur in the guinea pig's lung.

(a) Spasmodic contracture of the bronchial musculature unassociated with evident change in the pulmonary blood vessels.

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[5]Simonds: Jour. Am. Med. Assn., Nov. 8, 1919, lxxiii, 1437.
[6]Longcope: Jour. Exper. Med., 1913, xviii, 678; 1915, xxii, 793.
[7]Boughton: Jour. Immunol., 1919, iv, 213.
[8]Manwaring and Crowe: Proc. Soc. Exper. Biol. Med., 1916, xiv, 173.

(b) Spasmodic contracture of the pulmonary blood vessels. This reaction is usually accompanied by edema, and is commonly followed by a mild bronchial reaction.

(c) A condition which is referred to as pseudoanaphylactic, and which is described in this volume under the heading of anaphylactoid phenomena; namely, plugging of the pulmonary blood vessels with thrombi with consequent irritative contracture of the bronchial musculature.

An important experiment has been recently carried out by Manwaring and his associates.[9] This is described by them in the following words:

"If the lungs of a normal dog are perfused for about three minutes with Locke's solution, followed by Locke's solution containing from 0.25 to 1 per cent of horse serum, no recognizable pulmonary reaction takes place. The rate of perfusion flow remains constant on change from the Locke's solution to the dilute serum. When the tracheal clamp is released, the lungs collapse promptly and normally. No frothy fluid escapes from the trachea. If, however, the lungs of a sensitized dog are similarly perfused, very marked pulmonary reactions occur. For example, the rate of perfusion flow, which usually varies from 1,200 to 1,500 c.c. a minute in medium-sized dogs, is rapidly reduced to about 300 c.c. a minute. With smaller serum doses, a slight tendency to recovery is occasionally noted after the third minute.

"During this reaction, the lungs increase in size and take on a rubber-like consistency. When the tracheal clamp is released, practically no pulmonary collapse takes place. A large amount of clear, frothy fluid escapes from the trachea. If the perfusion is now continued, fluid continues to pour out of the trachea almost as rapidly as it escapes from the efferent cannula."

As Manwaring points out, the most striking feature of these reactions is the marked increase in capillary permeability thus demonstrated. They believe that increased specific capillary

[9]Manwaring, Chilcote, and Hosepian: Jour. Am. Med. Assn., Feb. 3, 1923, lxxx, p. 303.

permeability will ultimately be shown to be the dominant fundamental physiologic change in protein sensitization, to which all other anaphylactic reactions are secondary. This view, they believe, is in accord with clinical evidence.

According to Manwaring[9a] and his associates, when the partially inflated lungs of dogs sensitized to horse serum are perfused with Locke's solution containing from 0.25 to 2 per cent of horse serum, the following anaphylactic phenomena are noted. (a) A 75 per cent reduction in the rate of perfusion flow. (b) Complete immobilization of the lungs, the lungs showing no tendency to collapse when the tracheal clamp is released. (c) Marked perivascular edema. (d) The escape of large amounts of perfusion fluid from the trachea. The tracheal flow usually begins during the fourth minute, and reaches a maximum by the seventh minute. With medium sized dogs, about 1,000 c.c. of perfusion fluid escapes from the trachea by the end of the seventh minute.

In an attempt to determine the nature of the anaphylactically increased capillary permeability thus demonstrated, these observers added to the perfusion fluid various substances, and then made quantitative determinations of the substances in the fluid recovered from the trachea. They found that during the anaphylactic reaction the capillary endothelium offers no demonstrable resistance to the outward passage of hemoglobin and serum proteins. During the first seven minutes the passage of gelatin is retarded about 60 per cent, and that of gum arabic about 93 per cent. Starch and carbon particles are held back. There is also a reduction in the amount of fluid recovered from the trachea when gelatin, gum arabic or starch are added to the perfusion fluid.

The Rôle of the Liver in Anaphylaxis

In its passage through the intestinal villi, the portal blood comes in contact with many cells, whose function is to absorb and throw into the blood the various products of tryptic di-

[9a] Manwaring, W. H.; Hosepian, V. M.; and Thompson, W. L.; Quantitative Study of Anaphylactic Capillary Permeability, Jour. Am. Med. Assn., xxcii, p. 542, Feb., 1924.

gestion. Probably, also, proteolytic ferments are absorbed and carried to the liver. As pointed out by Falls,[10] it is also probable that, in consequence of decreased pressure and slower circulation, the carbon dioxide tension is greater in the portal than in the peripheral blood. Such a condition would tend to increase the rate of proteolysis. The rapid postmortem autolysis which takes place indicates that the liver is rich in ferments. These facts may explain the atypical reactions of anaphylaxis which are exhibited when antigen is introduced into the portal, rather than the peripheral, circulation. It is assumed by Abderhalden, as quoted by Falls, that the liver is a buffer, between the intestinal and general circulation, which protects the latter from the entrance of foreign protein which, when incompletely digested, would have poisonous potentialities.

Falls[11] has found that guinea pigs may be sensitized by intraportal as by other routes of injection of human serum. Anaphylactic shock may be obtained after subsequent injection by either intraportal or peripheral routes, but if the antigenic serum is injected into the portal circulation, larger doses are necessary to induce fatal shock.

An interesting series of experiments carried out by Manwaring[12] showed that exclusion of the spleen, stomach, kidneys, suprarenals, and ovaries from the circulation, had no effect upon the occurrence of anaphylactic shock. When, however, the liver was excluded from the circulation, none of the animals (dogs) reacted with anaphylactic shock to the injection of the serum antigen.

Manwaring and Crowe,[13] during the course of a series of experiments in which isolated organs were perfused with dilute solutions of foreign proteins, and the perfusion fluid subsequently tested for changes in toxicity by passage through isolated anaphylactic lungs, found that, although the perfusion of an antigenic protein through the liver of a normal

[10]Falls: Jour. Infect., Dis., 1917, xxii, 83.
[11]Falls: Jour. Am. Med. Assn., 1915, lxv, 524.
[12]Manwaring: Ztschr. f. Immunol., 18, 1911 (quoted by Zinsser).
[13]Manwaring and Crowe: Jour. of Immunol., 1917, ii, 511.

guinea pig did not alter the antigenic properties of the fluid, repeated perfusion of the liver from an anaphylactic animal invariably resulted in reduction in anaphylactic toxicity of the perfusing fluid. If the foreign protein is carried in anaphylactic blood and perfused through an anaphylactic liver, the fluid employed usually loses, almost completely, its power to produce an anaphylactic response when tested with the anaphylactic lung. "Furthermore, on repeated passages through the anaphylactic liver, the perfusion fluid also acquires a new power, that of causing an unusual relaxation in the pulmonary tissues. There is also a loss of the occasional power of the perfusion fluid to cause vasoconstriction. The detoxicating effect of the anaphylactic liver is, therefore, accompanied by, and is possibly due to, the explosive formation and liberation of vasodilator and bronchodilator substances. Similar findings were obtained by Simonds.[14] (Cunningham.[15])

The characteristic fall in arterial blood pressure, when hypersensitive dogs are injected with their specific antibody, does not take place in dogs which have been subjected to an Eck fistula, and are consequently dehepatized. From this fact, Manwaring and his coworkers,[16] have concluded that the characteristic fall in arterial pressure is in some way dependent on liver function.

"The theory we have proposed to account for this relationship assumes that the characteristic fall in arterial pressure is due to an explosive hepatic autointoxication, the formation or liberation of hepatic products having a histamin-like reaction on the extrahepatic vessels."

Effect of Anaphylactic Shock upon Coagulation of Blood

Coagulation of the blood in anaphylactic shock in dogs may be only delayed, or may be completely abolished (Weil). A similar change characterizes the blood of rabbits in anaphylaxis and of guinea pigs, if the shock be protracted. Weil

[14]Simonds: Jour. Infect. Dis., 1916, xix, 753.
[15]Cunningham: Jour. Dis. Children, 1920, xix, 400.
[16]Manwaring, Chilcote and Hosepian: Intestinal and Hepatic Reactions in Anaphylaxis, Jour. Am. Med. Assn., Sept. 10, 1921, lxxvii, 847.

believes that changes in the liver are chiefly concerned in the production of this alteration in the coagulability of the blood. He described experiments carried out according to a procedure introduced by Doyon, in which perfusion during life of the liver of a sensitized dog, with blood from a normal dog, showed that the coagulation time of the normal blood is prolonged to a remarkable degree when the specific antigen is added. The following experiment is quoted from Weil's contribution:[17]

October 5. 5 c.c. horse serum subcutaneously.
October 8. 5 c.c. horse serum intravenously.
October 29. Doyon method.
12.20 Carotid-portal circulation established.
12.23 Cava blood, clotting time two minutes.
12.25 3 c.c. of horse serum into tubing.
12.34 3 c.c. of horse serum into tubing.
12.53 Cava blood remains unclotted after twenty-four hours.

Effect of Anaphylactic Shock upon Temperature

Immediate anaphylaxis is characterized by a pronounced lowering of body temperature gradually falling to 20° centigrade and ending in death. If, however, the onset of symptoms be more gradual, an elevation in temperature occurs. Not infrequently in man the onset of hyperpyrexia is accompanied by chills.

Effect of Anaphylactic Shock upon Leucocytes

In rapidly fatal cases a leucopenia is noted; in those which progress more slowly, there occurs a primary depression of the number of white cells which is followed by an increase and is accompanied by increased activity of the myelogenous tissues. This is evidenced by the appearance in the circulating blood of immature forms of the polymorphonuclear leucocyte.

[17]Weil: Studies in Anaphylaxis: Anaphylaxis in Dogs. A Study of the Liver in Shock and in Peptone Poisoning, Jour. Immunol., 1916-17, ii, 525.

Depletion of Complement in Anaphylaxis

Decrease in the complement-content of the blood during anaphylactic shock has been noted by Michaelis and Fleischmann,[18] Sleeswijk,[19] Friedmann,[20] Friedberger and Hartoch,[21] and Scott.[22]

Francioni[23] states that a lack of complement is found in serum sickness in man. This corresponds to the findings of Ehrlich and Morgenroth and Moreschi, who, after the first injections of rabbits with serum, found the complement diminished between the eighth and tenth days. Sleewijk[24] made an exact examination of the alexin content after anaphylactic shock. Thirty minutes after injection, the amount of alexin falls to a minimum. After two hours it is, again, normal in amount.

Increase in Blood Nitrogen in Anaphylaxis

In anaphylaxis in guinea pigs, as well as after peptone poisoning, there is a considerable increase in noncoagulable urea nitrogen in the blood, as well as a slight increase in amino-nitrogen, although it is not known whether this comes from the tissues or from the antigen-antibody reaction in the blood. The former seems more probable (Hisanobu[25]).

Lymphagogue Reactions in Dogs

Quantitative measurements by Calvary[26] have shown that anaphylaxis in dogs is accompanied by a marked increase of the lymph flow (seven times the amount observed in normal dogs in the same time). He was able to show by controlling the blood pressure with barium chlorid that this lymphagogue action is not directly dependent upon the low pressure.

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[18]Michaelis and Fleischmann: Med. Klin., 1906, No. 1.

[19]Sleeswijk: Münch. med. Wchnschr., 1907, No. 34.

[20]Friedmann: Ztschr. f. Immunitatsf., Orig., 1909, ii, 591.

[21]Friedberger and Hartoch: Ztschr. f. Immun. Orig., 1909, iii, p. 581.

[22]Scott: Jour. Path. and Bact., 1909, iv, 147.

[23]Francioni: Riv. di clin. Pediat., 1908, vi, 321: Sperimentals, 1904, p. 767; Riv. di clin. Pediat., 1907, v, 606.

[24]Sleewijk: Ztschr. f. Immun. u. exper. Therap., 1909, ix, 133.

[25]Hisanobu: Am. Jour. Physiol., 1920, i, 357.

[26]Calvary: Münch. med. Wchnschr., 1911, No. 13. (Quoted Zinsser p. 369.)

Nonstriated Muscle Reaction in Vitro, Schultz-Dale Reaction

An important form of the anaphylactic reaction has been extensively studied, by means of a technic known as the Schultz-Dale method. This method consists in suspending the uterus (or strip of intestine) from a sensitized guinea pig in a bath of Locke's fluid, and of tracing the muscular contractions on a revolving drum. Prior to suspension of the uterus in Locke's solution the uterus, along with the viscera in the hind part of the animal, is perfused for one hour with Locke's solution. At the end of this period, Dale states that the muscle has lost its pink color, and believes that it contains no more serum than is contained in washed red blood cells, such as are used in ordinary hemoglobin experiments.

Schultz[27] has shown that horse serum induces contraction in excised portion of guinea pigs' unstriped muscle, also that if the muscle employed be from a sensitized animal, the reaction which occurs is more marked. Dale[28] confirmed Schultz' experiments, and has shown that the virgin guinea pig uterus from a sensitized animal reacts by powerful contractions to dilutions of protein antigen of one to 100,000 or even one to 500,000. In such dilutions sera do not induce contractions in nonsensitized muscle.

Dale[29] considers that the response on the part of the uterus from a sensitized guinea pig, when the organ is brought in contact, outside the body, with its specific antigen, is not merely an exaggeration of a normal reaction which plain muscle gives to fresh sera in general. When purified protein, such as precipitated sera, globulin, or crystallized egg albumen, is employed, as antigen, no reaction is obtained when the normal plain muscle is employed; but a typical reaction is obtained if the muscle be from an anaphylactic animal. One dose of the specific antigen, in sufficient concentration to produce maximal response to the anaphylactic uterus, completely desensitized the

[27]Schultz: Jour. Pharmacol and Exper. Therap., 1912, iii.
[28]Dale: Jour. Pharmacol. and Exper. Therap., 1912-1913, iv, 167.
[29]Dale: Jour. Pharmacol. and Exper. Therap., 1912-13, iv, 167.

latter. Either normal or anaphylactic plain muscle gives repeated response to successive large doses of a normally toxic serum, but this phenomena is not an anaphylactic response.

Sensitiveness of washed unstriped muscle is seen in passive, as in active, anaphylaxis, and is obtained whether the serum producing passive sensitization is obtained from a sensitive or from an immune guinea pig. After sensitized muscle has been desensitized, mere contact with a not too great dilution of sensitive serum for some hours results in its again becoming anaphylactic. Dale was unable to sensitize normal plain muscle in this way. He found that perfusion of a normal uterus for five hours with diluted serum from immune guinea pigs produced a decided passive sensitization.

Dale has also noted that bronchial spasm of the anaphylactic guinea pig lung is produced with apparently undiminished vigor when isolated lungs are perfused with Ringer's solution, which contains the antigen.

Dale[30] is of the opinion that the anaphylactic response of isolated plain muscle strips differ fundamentally from that which occurs in the uterus from normal animals, when it is treated with foreign serum. His experiments indicate that this is the case, although it must be pointed out that it is quite conceivable that the reaction which occurs is due to irritation or disturbance with metabolic activity in consequence of a substance which is alike present in toxic sera and produced as the result of the interaction of anaphylactic antibody and antigen.

With reference to the rapid rise of tonus when the plain muscle strip from an anaphylactic animal is treated with its specific antigen, Dale remarks: "The effect can probably be accounted for by the mere initiation of those changes in the state of aggregation of the colloidal particles, which, when antibody and antigen are present in appropriate proportion, and sufficient time is allowed, result in the formation of a visible precipitate. It is not, however, necessary to assume the

[30]Dale: Jour. Pharmacol. and Exper. Therap., 1912-13, iv, 167.

identity of precipitin and anaphylactic antibody; if they be identical, as seems very probable, it is not necessary that the antibody should be present in such proportion as to give with the antigen a visible precipitate. All that is needed is that the antibody should have such a specific physical relation to the antigen that, when the two meet, a disturbance of the conditions of colloidal solution is set up in the muscle fiber.''

CHAPTER IX

TRANSFERRED ANAPHYLAXIS

That the anaphylactic (first order) antibody is present in the serum of sensitized animals is proved by means of experiments of transferred sensitization. If 3 c.c. of serum be collected from a sensitized guinea pig, and injected into a normal pig, the latter animal may be proved to have been passively rendered hypersensitive. Subsequent injection of antigenic protein, into the passively sensitized pig, is followed by characteristic manifestations of anaphylaxis.

This phenomenon is known as transferred anaphylaxis, and is of the utmost importance since, in addition to proving that the anaphylactic antibody is present free in the serum of sensitized animals, it has rendered quantitative experiments possible. Passive sensitization may be accomplished with any serum that contains antibodies. The quantitative power of such a serum to convey passive sensitization is in direct proportion to its antibody concentration (Zinsser).

Anderson and Frost[1] found that 3 c.c. of the serum of a guinea pig sensitized with a single injection of horse serum, is approximately the minimum amount that will constantly passively sensitize 250 gram guinea pigs, so that they will react definitely to an intraperitoneal injection of 5 c.c. of horse serum twenty-four hours later. They also noted that serum of tolerant guinea pig contains three or four times as much of the anaphylactic antibody as does the serum of the guinea pig rendered hypersensitive by a single dose of antigen. They further discovered that the serum of a rabbit, treated with frequent large injections of horse serum, contains more free sensitizing antibody than does the serum of highly sensitive guinea pigs.

[1]Anderson and Frost: Trans. Cong. Am. Phys. and Surgs., 1910, viii, 414.

These experiments have been repeatedly confirmed. It has thus been shown that an animal of a species, such as the rabbit, which is comparatively insusceptible to immediate anaphylaxis, may yet carry in its blood even more first order antibody than an animal of another species, such as the guinea pig, which is highly susceptible.

It has been found difficult by all observers to induce passive anaphylaxis if the transferred serum and the antigenic protein are injected simultaneously, or immediately, following one another. The most dependable results are obtained if an interval of twenty-four hours, more or less, be allowed to elapse, between the introduction of the sensitizing serum and the antigen. It is still maintained by many observers that this period, which is known as the incubation period *must* elapse between the injection of the sensitizing serum and the introduction of the toxic dose, in order that anaphylactic shock may be exhibited. On the other hand, experiments appear to have proved that the incubation period is not always necessary.[2]

Doerr and Russ,[3] Friedberger,[4] Richet,[5] Biedl and Kraus,[6] Weill-Halle and Lamaire,[7] have been successful in producing immediate shock by the injection of suitable mixtures of antigen and antibody. Their results have been confirmed by the author.

The author,[8] in 1911, published the results of experiments which proved the possibility of immediate symptoms of intoxication following the introduction of transferred serum and antigenic protein without an intervening period of time. The fact that it has been possible to induce fatal reactions in this manner indicates that the fixation of receptors is not an essential step in the phenomena of transferred anaphylaxis.

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[2]It is proper to point out at this point that these experiments have not found general acceptance among immunologists. The subject is discussed more argumentatively later in this volume.

[3]Doerr and Russ: Studien über Anaphylaxie, II. Ztschr. f. Immunol., 1909, iii, No. 2, p. 181.

[4]Friedberger: Weit. Mitteilung über Eiweissanaphylaxie, IV, Ztschr. f. Immunol., Orig., V. 4, Heft 5- 636.

[5]Richet: Compt. Rend. de la Soc. Biol., 1909, lxvi, No. 18.

[6]Biedl and Kraus: Ztschr. f. Immunol., 1910, iv, No. 1 and 2.

[7]Weill-Halle and Lamaire: Compt. Rend. de la Soc. de Biol., 1908, lxv, 141.

[8]Gurd: Jour. Med. Research., 1914, xxxi, 205.

For the strain of guinea pigs used in these experiments the minimal lethal toxic dose of sheep serum, fourteen days after a sensitizing dose of 0.01 c.c. of sheep serum, was 0.25 c.c. If a sensitizing dose of 0.05 c.c. was used the minimal lethal toxic dose was 0.20 c.c. As an arbitrary lethal toxic dose, 0.20 c.c. was adopted as one unit.

The sensitizing serum which was employed was derived from rabbits which had received repeated doses of sheep serum over several months. It was established that 0.6 c.c. of this serum

GUINEA PIG NO.	WEIGHT	PASSIVE SENSITIZATION	TOXIC INJECTION	RESULT	AUTOPSY
135	290 gm.	1.0 c.c.	.5 c.c.	Immediate onset of marked symptoms; scratching; irritability; dyspnoea; partial recovery; dead in A. M.	Lungs moderately distended, with haemorrhagic patches.
119	225 gm.	.8 c.c.	.6 c.c.	Immediate onset of very marked symptoms; convulsions; recovery.	
113	200 gm.	.5 c.c.	.5 c.c.	Immediate onset of very marked symptoms; complete paralysis and convulsions. Apparently dying but recovered.	
143	325 gm.	1.6 c.c.	.5 c.c.	After five minutes onset of marked symptoms; culminating in death 1 hr. after injection.	Marked distention of lungs, with numerous subpleural hemorrhages.

was the smallest amount that regularly conferred passive sensitiveness upon normal guinea pigs, when given forty-eight hours prior to a dose of one and one-half units of sheep serum.

In the actual performance of the experiments two and one-half to three units of the antigen sheep serum (viz., 0.5 to 0.6 c.c.) were employed as intoxicating doses.

Control experiments which were published at that time show that it is obvious that relatively large doses of sheep serum do produce slight evidence of irritation in normal pigs. These manifestations are not comparable to those tabulated below.

Protocol.—Guinea pigs were injected, with immune rabbit serum, into one jugular vein and immediately thereafter with sheep serum into the same or the opposite jugular vein.

Transferred Anaphylaxis from Mother to Offspring.—The young born of mothers who possess a marked degree of hypersensitiveness may be proved to present the same anaphylactic state upon subsequent injections with a toxic dose of the specific protein against which the mother had been sensitized.

As with other immune bodies the anaphylactic substance is secreted in the milk so that nursing offspring develop passively a mild degree of hypersensitiveness if the mother be treated immediately after their birth (Wells).

CHAPTER X

DESENSITIZATION

The injection of a sublethal dose of foreign protein, intravenously, results in the immediate onset of symptoms of anaphylaxis. The animal, however, rapidly recovers, and after a lapse of ten or fifteen minutes is apparently well. It is further noted that subsequent injections, during a period of two to five days, of lethal doses of antigen prove the animal to be nonsensitive to the foreign protein, nor is the serum of such an animal capable of transferring hypersensitiveness to another animal. The animal that has been thus rendered nonsensitive is said to have been desensitized.[1] This refractory condition lasts for a period of several days. It is followed by a gradual return of hypersensitiveness, or by the development of the tolerant state. Not only may an animal be desensitized by the sublethal intravenous introduction of the foreign protein, but also by means of injections made intraperitoneally or subcutaneously, even though no frank anaphylactic symptoms are manifest. It is noteworthy, moreover, that the dose of foreign protein need not be so large as the minimum lethal dose; a relatively small quantity of the antigenic material is sufficient to render the animal refractory.

It is possible by means of the intravenous introduction of 0.002 centimeters of sheep's serum, into an animal which has been so sensitized that a dose of 0.02 cubic centimeter of serum administered intravenously is followed by fatal "shock," to absorb or exhaust the available anaphylactic antibodies so that subsequently the lethal dose may be administered

[1]The foregoing phenomenon of desensitization has been designated by the term antianaphylaxis (Besredka, Rosenau and Anderson, and others). The author, along with others, believes that such an employment of this expression is not the most suitable. In the author's opinion the term antianaphylaxis, if used at all, should be reserved to designate the condition of tolerance or "immunity" which is developed by the tissues following repeated injections of antigenic proteins.

without the exhibition of any reaction whatever. In a similar way, the hypersensitive animal may be subjected to the administration of 0.01 c.c. of sheep's serum into the subcutaneous tissues with the development simply of a mild reaction. After recovery from such a reaction the animal is often found to be not sensitive to the intravenous injection of one or more lethal doses.

In experiments of passive anaphylaxis the addition of even very minute quantities of the specific protein are sufficient to inactivate the sensitizing serum, so that subsequent attempts to induce shock became failures.

The phenomenon of desensitization is evidently due to the fact that the anaphylactic bodies are satisfied, exhausted, or neutralized by the preliminary admixture of the protein antigen either *in vivo* or *in vitro.* The presence of quantitative relationships between the antigen and anaphylactin (first order antibody), in this reaction, proves that the latter is subject to the same laws of absorption as are other immune bodies.

Isolated muscle strips exhibit the same phenomenon of desensitization immediately after exposure to the specific antigen as does the living animal.

The same quantity of specificity characterizes desensitization as anaphylactic shock. If the guinea pig has been sensitized to two different protein antigens and recovers from the shock induced by the injection of one of them it becomes desensitized to this antigen, but will still react to the second protein. Wells has taken advantage of this phenomenon, and has found it reliable in determining the property of protein preparations. He has noted, however, that the second reaction has seldom been so severe as it would have been if it had been a primary reaction. This is due, he believes, at least in part, to exhaustion of the reacting mechanism.

The dog, the rabbit, and man are subject to desensitization as the result of an injection of a sublethal dose of the antigen in the same way as is the guinea pig. As data is gradually accumulated, it is evident that the phenomenon of desensitiza-

tion, or exhaustion of antibodies (first order bodies), is characteristic of anaphylaxis in all animals.

Much of the pioneer work regarding desensitization has been carried out by Besredka. He sums up his opinion in the following way: "We can prevent anaphylaxis from occurring by the following different methods of injection,—oral, rectal, subcutaneous, intraperitoneal, intracerebral, intrathecal and intravenous. The oral method is the least practical of all, because it requires at least one or two days before antianaphylactic immunity (desensitization) is established. The rectal method is more prompt in action, but it is subject to some risks, the reabsorption of the antigen by the mucosa being delayed according to individual idiosyncrasy and the nature of the antigen. The intraperitoneal and intracerebral methods—above all, the latter—confer immunity (desensitization) in a very short time, varying from a few minutes to an hour at the most. This immunity is the most effective and reliable; but it is to be understood that these methods may be impracticable in the case of man. There remains vaccination by the subcutaneous, intrathecal, and intravenous routes. From these routes the physician will have to make his choice."[2]

[2]Besredka-Gloyne: Anaphylaxis and Antianaphylaxis, 1919, C. V. Mosby Co.

CHAPTER XI

TOLERANCE

An animal may be insusceptible to anaphylactic shock following the injection of a foreign protein for one or other of three reasons.

1. A normal animal because there are no antibodies present in the tissues to react with the antigen.

2. A desensitized animal because of exhaustion of the antibodies.

3. A tolerant animal; that the tolerant animal differs from both the normal animal and the desensitized animal is evident. In the following pages the author attempts to indicate the nature of the protective mechanism.

If an animal, which has received several sublethal doses of protein antigen, be tested by means of a dose of antigen, which has proved to be fatal to animals which have been rendered anaphylactic by means of a single small dose of antigen, no symptoms of tissue irritation are exhibited. If such an animal be bled and a small quantity of its serum, 1 to 3 c.c. be introduced into the peritoneal cavity, or by any other route, of a normal animal, it is found that the normal animal has been passively sensitized. If such a guinea pig be given an ordinary lethal dose of protein antigen intravenously it dies in typical anaphylactic shock.

Obviously, therefore, the serum of the animal which has received in addition to a sensitizing dose, repeated sublethal injections of protein antigen, is not in the same state as is the normal animal, nor the desensitized animal. This is proved since, although the repeatedly injected animal is itself tolerant to normal lethal doses of antigen, its serum confers passive hypersensitiveness upon the normal animal.

Certain facts have been established which have, at least, an

indirect bearing upon this subject. It will be of value to review these at this time. Normal guinea pigs, if injected with small quantities, e.g., 0.05 c.c. of heterologous serum protein, become markedly hypersensitive to reinjection with the same antigen after a lapse of fourteen days. If a second injection of the foreign serum be made, either before complete hypersensitiveness has developed or in a quantity too small to cause death of the animal, the anaphylactic state does not develop in a manner exactly similar to that which occurs in the normal animal which has received but one injection. The difference noted consists in the ability of the second type of animal to withstand larger doses of foreign serum. Such an animal can, however, be proved to be highly anaphylactic if larger doses of antigenic protein be employed. It is thus possible to prove that animals treated in the same manner (by repeated sublethal injections of antigen) are relatively tolerant, and, at the same time, markedly hypersensitive. The more frequently the animal has been treated with sublethal doses, and the larger the doses which have been introduced, the more marked is the animal's insusceptibility to intoxication (tolerance), and the more difficult is it to prove hypersensitiveness. It must be noted, however, that it is not possible to render an animal sufficiently tolerant that it is able to withstand more than three to five times the usual toxic dose of antigen.

In a series of experiments performed by the author[1] it was was found that, whereas the injection of 0.5 c.c. of serum from a rabbit, which had received repeated doses of sheep serum, served to passively sensitize normal guinea pigs so that anaphylactic shock developed when the guinea pig was injected with 0.45 c.c. of antigenic serum, the injection of 2.75 c.c. of the same rabbit serum rendered the recipient guinea pig tolerant to a like dose of antigen.

These experiments show the protective effect of large, as compared with the sensitizing effect of small, doses of immune rabbit serum upon guinea pigs when an interval of time elapses

[1]Gurd: Jour. Med. Research, 1914, xxxi, 205-222.

PROTOCOL

GUINEA PIG NO.	WEIGHT	FIRST INJECTION	INTERVAL	SECOND INJECTION	RESULT
109	225 gm.	.6 c.c. I.R.S., i.p.	24 hr.	.35 c.c. S.S., i.v.	Marked symptoms. Recovery.
110	225 gm.	.6 c.c. I.R.S., i.p.	24 hr.	.34 c.c. S.S., i.v.	Typical anaphylactic. Death 6 minutes.
103	225 gm.	4 c.c. I.R.S., i.p. 12 hr. later, 4.5 c.c. I.R.S., i.p.	24 hr.	.5 c.c. S.S., i.v.	Slight Malaise.
99	210 gm.	1 c.c. I.R.S., i.p.	7 days	.3 c.c. S.S., i.v.	Very severe symptoms.
100	200 gm.	1 c.c. I.R.S.	7 days	3.5 c.c. I.R.S., i.v. .4 c.c. S.S., i.v.	Very slight symptoms.
147	210 gm.	5 c.c. I.R.S.	24 hr.	.5 c.c. S.S., i.v.	Typical death 4 minutes.
148	215 gm.	.75 c.c. I.R.S.	24 hr.	.5 c.c. S.S., i.v.	Typical death 3 minutes.
149	205 gm.	2.5 c.c. I.R.S., i.p.	24 hr.	.5 c.c. S.S., i.v.	Marked symptoms. Recovery.

I.R.S.—Immune rabbit serum.
i.p.—Intraperitoneal injection.
i.v.—Intravenous injection.
S.S.—Sheep serum.

between injection of the transferred serum and the introduction of the toxic dose.

Protocol. Experiment No. 138

Guinea pigs of 200 gm. weight were employed. These received intravenous injections of immune rabbit serum and antigenic protein (sheep serum) within two to four minutes of one another.

GUINEA PIG NO.	RABBIT SERUM	SHEEP SERUM	RESULT
113	.5 c.c., i.v.	.5 c.c., i.v.	Four minutes onset of very severe symptoms. Scratching of nose; dyspnea; convulsions, paralysis. Thought to be dead, but recovered.
111	.9 c.c., i.v.	.5 c.c., i.v.	Four minutes onset of moderate symptoms. Dyspnea; mild convulsive seizures.
112	2.75 c.c., i.v.	.5 c.c., i.v.	No symptoms.

These experiments are interpreted by the author as proving the presence, in the circulating blood of immune animals, of bodies which are potent to induce the hypersensitive state when introduced into normal animals and, also, of bodies which if injected in sufficient quantities are able to render normal animals passively tolerant (immune).

Thomsen[2] states that a sensitizing dose of 0.004 cubic centimeters of serum produced a maximum sensitization more quickly than 0.1 cubic centimeter, although the maximum degree of sensitization is the same with each dose.

The explanation of this fact is apparently a simple one, namely, that as long as there remains in the tissue of the injected animal a remnant of the introduced antigen, the anaphylactic antibodies are exhausted as soon as they are produced. The animal thus remains desensitized, even though its tissues are actively producing the anaphylactic antibodies. When very large doses of antigen are given tolerance may be engendered. Under these conditions anaphylaxis is not exhibited when minimal toxic injections of protein antigen are employed, until a sufficient period has elapsed for the second order antibody to diminish in quantity.

Certain experiments reported by Vaughan (loc. cit., page 173) prove that it is possible to develop, to a limited degree, at least, the production of the protective or immunity conferring substance without rendering the animal sensitive. According to Vaughan it is possible to so treat an animal by the repeated injections of the poisonous protein split product, prepared

[2]Thomsen: Ztschr. Immunität., 1917, xxvi, 213.

according to his method,[3] that a state of tolerance is developed as the result of which the animal is able to "bear three or four times the minimum lethal dose," and that furthermore, such an animal is not sensitive to the whole protein from which the poison is recovered.

Zinsser and Dwyer have reported experiments regarding tolerance to typhoid anaphylatoxin. Working with typhoid anaphylatoxin they found that guinea pigs, treated with sublethal doses of anaphylatoxin, developed a tolerance which enabled them to resist 1½ to 2 units of poison. Such tolerance is exhibited within three days and lasts to a slight degree, for as long as two months. The tolerant state did not seem to be strictly specific, in that typhoid anaphylatoxin seemed to produce a moderate tolerance to prodigiosus anaphylatoxin.

A certain degree of tolerance may also be induced by the primary administration of large doses of antigen. Rosenau and Anderson[4] and others have published experiments in which it is shown that, whereas animals become hypersensitive within eight or twelve days after the administration of small doses of serum (0.01 c.c. or less), if the primary injection consists of 3 or 4 c.c. of an antigen serum it takes several weeks before the animals become markedly anaphylactic.

Antisensitization

Antisensitization is a phenomenon somewhat similar to tolerance, described by Weil.[5] If a guinea pig be given a single dose of serum, several days before a sensitizing dose of serum from a rabbit immune to a foreign protein, the usual passive sensitization does not take place. This is explained by the development in the guinea pig of antibodies to the rabbit serum, which protect the guinea pig's tissues from the antibodies of the immune rabbit serum. In proof of this conclusion is the fact that such preliminary injection with rabbit serum does not prevent passive sensitization with the serum of a guinea pig immunized to foreign protein.

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[3]See p. 133.

[4]Rosenau and Anderson: U. S. Pub. Health and Marine Hosp. Ser. Lab. Bull., 1908, xlv, quoted by Zinsser.

[5]Weil: Ztschr. Immunität., 1913, xx, 199.

CHAPTER XII

NATURE OF ANAPHYLACTIC ANTIGEN[1] (ANAPHYLACTOGEN)

"There is no doubt that any soluble protein which can act as an antigen in other immunologic reactions can act as an anaphylactogen, and the only soluble proteins as yet found not to be antigenic are those which are, from the chemical sense, incomplete proteins." (Wells.) Those that are not antigenic include such proteins as have a very small variety of amino acids, notably the protamines and histones; also the complexes of these with nonprotein radicals, notably, the nucleo-proteins and hemoglobins. Gelatin, on the other hand, although a very complex and soluble protein, has no antigenic power, whether tested by anaphylaxis or by more sensitive immunologic methods. The chief difference between gelatin and the ordinary proteins that do exhibit antigenic properties is a deficiency in aromatic amino acids. It is, therefore, assumed by Wells that aromatic amino acids must be present.

In discussing the amino acid constituent of antigenic proteins, Wells says: "There exist proteins which lack one or more of the amino acids which are commonly present in typical proteins, yet which are strongly antigenic (e.g., zein from corn, which lacks glycine and tryptophane, gliadine of wheat and rye, or hordein of barley, which contain no glycerin or lysin and very little arginine or histidine). The fact that these last three proteins, which are so extremely poor in diamino acids, are potent, while the protamines which consist chiefly of diamino acids, are inert, suggests that these three diamino acids are not of importance in the anaphylactic activity of proteins, but since no protein is known which does not

[1]The facts presented in this chapter upon the anaphylactic antigen are equally applicable to other antigens.

contain either histidine or arginine, we cannot prove this point as it seems to be proved for lysin.'' (Wells.[2])

Solubility of the protein is essential, in Well's opinion, since, although insoluble protein may eventually be brought into solution in the animal body, the process is too slow to bring about reactions, and also it is probably accompanied by disintegration of the protein molecule. Heat, of degrees that do not disintegrate the proteins, affects them only to the extent that it makes them insoluble. There are, however, only a few known proteins that are not made insoluble by heat, and these, except gelatin, are antigenic despite boiling. In this group are casein, ovomucoid, the so-called proteoses of plant seeds, beta-nucleo proteins, and perhaps the capsular substance of pneumococci.

As Besredka has pointed out, if soluble antigens be diluted they are rendered much less susceptible to coagulation. When diluted the antigenic properties of the protein are not destroyed by moderate degrees of heat.

Trypsin digestion, as might well be expected, gradually, though slowly, destroys the sensitizing power which latter runs parallel to the remnants of coagulable protein.

Compound proteins produce anaphylaxis if they are soluble, and if the protein constituents are themselves antigenic, but apparently not when the protein radical is a nonantigenic histone or protamine. Thus, alpha-nucleoproteins and hemoglobin are nonantigenic, while nucleins, beta-nucleoproteins and hemocyanin are antigenic (Wells).

In order that acute anaphylactic shock may be readily produced, it is essential that the protein be soluble. On the other hand, in order that symptoms and signs of tissue irritation may be induced, solubility of the antigen is not necessary. The author has attempted elsewhere to point out that, whereas, if a soluble protein be introduced into the blood stream, or into

[2]Wells and his associates have performed many experiments and have recorded their observations regarding the nature of anaphylactic antigens (anaphylactogens). For the most part the material presented in this section is abstracted from the publications of these authors. For a more complete consideration of the subject, the reader is referred to Well's *Chemical Pathology* (Saunders, Philadelphia), or The Present Status of the Problems of Anaphylaxis, *Physiological Reviews,* 1921, 1, 44.

other tissues in which its absorption into the blood stream is readily accomplished, general manifestations of irritation or intoxication take place in an explosive fashion; the injection of either a less soluble antigenic protein, or by a route by which its absorption is less rapidly brought about is followed to a less marked degree by constitutional symptoms, but by more marked evidence of focal irritation. It is to this focal reaction to the introduction of proteins that the term "allergy," in the author's opinion, should be restricted. Anaphylaxis then, from this point of view, described the explosive generalized symptoms of constitutional intoxication when the sensitized animal is subjected to an injection by the same protein to which its tissues have previously been exposed, whereas allergy designates the phenomena which occur focally in the tissues at the site of injection of a less rapidly absorbable protein.

The racemized proteins[3] of Dakin are substances which in every way resemble simple proteins, except for their diminished optical activity. They also exhibit the property of being resistant to proteolytic enzymes, and are not metabolized when fed to, or injected under the skin of, experimental animals. Likewise, they do not act as antigens. Presumably, as pointed out by Wells, these characteristics are due to changes in structural configuration. Observations by Ten Broeck[4] with the racemized protein of egg albumin supports the theory that it is the breaking down in the tissues of an injected protein that causes the production of antibodies.

[3]A 20 per cent solution of Merck's soluble egg albumin in N/2 sodium hydroxide was incubated at 37° C. for three weeks. During this time the optical rotation gradually fell until it reached a constant value. The racemized protein, after neutralization with sulphuric acid was then salted out by saturating the solution with ammonia sulphate. The precipitate was filtered off, suspended in a little water and dialyzed in the presence of a little toluene to free it from salts. The racemized egg albumin went into solution in the dialysis tube and was precipitated from this solution by the addition of alcohol.

The racemized egg albumin so obtained was filtered off and dried, and formed a white powder resembling the original protein. It gives the typical protein reactions (biuret, etc.) and differs chemically from the original substance only in its optical properties. Its aqueous solution coagulates on heating, as in the case of ordinary egg white. The reactions for tyrosine, tryptophane and cystine are all positive. Like racemized casein and caseose, it is unaffected by the proteolytic enzymes of the digestive tract. (Dakin: Quoted Ten Broeck.[4])

[4]Ten Broeck: Jour. Biol. Chem., 1914, xvii, 369.

In discussing the question whether anything but an entire protein molecule can act as an anaphylactogen, Wells, after discussing the uncritical nature of much of the work which has been done upon this subject, concludes that "there still remains no satisfactory proof that anything except protein can act as an anaphylactogen."

Regarding the question as to whether lipoids have antigenic activity the matter must, at the present time, be considered unproved, although the fact that lipoid substances bind alexin is very suggestive.

Anaphylactoid reactions take place following the administration parenterally of certain drugs and chemicals. Practically all observers, who have employed salvarsan in any considerable number of cases, have noted such reactions. Wolff-Eisner suggests the theory that such reactions are due to an alteration of the recipients own tissue protein in such a way that they act as foreign proteins, and as such, are antigenic. Of the hypotheses brought forward to identify these drug reactions as anaphylaxis, this is the most suggestive. Up to the present time no direct positive evidence has been forthcoming. It has been, hitherto, impossible to produce passive anaphylaxis with the serum of persons hypersensitive to drugs, nor in Wells' opinion, have convincing experiments, proving active sensitization in guinea pigs, been published. The author is of the opinion that although no direct proof is available identifying the anaphylactoid reactions to the parenteral introduction of drugs as true anaphylaxis, the experimental difficulties in procuring such proof are such that failure must not be accepted as final.

Abderhalden has calculated that the 20 amino acids that are present in proteins could form at least 2,432,902,008,176,640,000 different compounds.

The biological importance of the data obtained by the use of the anaphylactic reaction has been summed up by Osborne,[5] as follows: "From these facts it is evident that structural differences exist between very similar proteins of different

[5]Osborne: Harvey Lectures, 1910-11.

origin, and it is interesting to note that chemically identical proteins, apparently, do not occur in animals and plants of different species, unless the latter are very closely related biologically. In this respect the proteins are in marked contrast to the other constituents of plants and animals, for not only do the same sugars and fats occur in many species of plants and animals, but many of these are common to both forms of life. The morphologic difference between species find their counterpart in the protein constituent of these tissues.''

Since gelatin, which is relatively poor in aromatic radicles, does not sensitize, and since it does not yield any toxic substance when injected into animals, it has been suggested that the anaphylactic reaction to proteins may depend upon the aromatic radicles, possibly through their separation from the remainder of the protein molecule.

The proteins definitely recognized as being capable of inducing anaphylaxis are, the albumins, globulins, nucleoproteins, and the albumoses. Among these substances are included a large number of bacterial derivatives. The greater amount of work upon bacterial proteins has been carried out with tuberculoprotein, particularly by Baldwin, Kraus, and their coworkers at the Saranac Lake Laboratories.

Specificity of the Anaphylactic Reaction

The phenomenon of anaphylaxis is characterized by the exhibition of the same specificity as that which exists between antibody and antigen in other immunity reactions. Incidentally, it may be stated that the reaction has been made use of, on account of its specificity, to differentiate various types of proteins which have not been subjected to identification by methods of chemical analysis. Doerr and Russ[6] found that guinea pigs of the same weight, passively sensitized in the same way to sheep serum, reacted to reinjection of sera from other sources in the following minimal doses: (Hektoen.)

[6] Doerr and Russ: Ztschr. f. Immunol., 1909, iii, pp. 181 and 706.

Sheep	Serum	0.004
Goat	"	0.004
Beef	"	0.01
Swine	"	0.6
Human	"	1.0
Horse	"	2.0
Chick	"	no reaction

These experiments demonstrate species specificity in anaphylactic shock, as in other immunity reactions. This specificity is equally well marked in the case of bacterial derivatives.

An interesting point to which attention has been drawn by Uhlenhuth,[7] and confirmed by Wells and others, is that not only does species specificity exist in the manner suggested above, but that there is, also, an important organ specificity. These observers found that, if an animal be sensitized to lens protein, not only will it react to the homologous lens protein but, also, to the lens substance derived from the same species or even its own lens. Recent experiments by Hektoen[8] have confirmed in a striking fashion this organ specificity or lens protein. Hektoen has made the suggestion that this may be an explanation of the phenomenon of sympathetic ophthalmia.

Another observation in this respect which may be of great significance in the study of eclampsia and other abnormal physiologic states characterizing pregnancy, is that the fetal tissue, placenta and amniotic fluid may cause anaphylaxis in members of the animal's own species. Heide, whose experiments have been repeated by Rongy,[9] has found that injections of fetal serum may bring on labor, if injected into pregnant women shortly before term. In addition to uterine contractions there frequently occur chills, nausea and vomiting, and precordial oppression or pain.

The chief obstacle, heretofore, met with in determining accurately the specificity of the reaction, has been the difficulty of obtaining pure material. Wells and Osborne have carried out pioneer experiments in this direction. They separated from

[7]Uhlenhuth: Ztschr. f. Immunol., 1910, iv.
[8]Hektoen: Jour. Am. Med. Assn., July 2, 1921.
[9]Rongy: Am. Jour. Obst., 1912, lxvi, p. 1.

egg white substances of definite chemical characteristics, namely, ovomucoid, ovoglobulin, ovalbumin, ovovitellin (from yoke), and nucleoalbumin, and found that animals sensitized to these different components reacted much more constantly and readily to the pure material, by means of which they were sensitized, than to either the total egg white, or to the other constituents. Wells found that ovoglobulin prepared by the treatment of egg white with $(NH_2)SO_4$ to half saturation sensitized in doses as small as 0.00001 gram, and that guinea pigs reacted to a toxic dose of 0.001 gram. Employing the recrystallized ovalbumin they found that one-twentieth of a milligram sensitized, while 1/1,000,000 gram prepared for a second lethal dose of one-twentieth of a milligram. It may be mentioned that these experiments of Wells, in addition to numerous others along other lines, prove that the same material is active in sensitizing and in provoking toxic symptoms.

Well's experiments have led him to the conclusion that, "the entire protein molecule is not necessarily involved in the specific character of the anaphylactic reaction, but this is developed by certain groups contained therein, and that one and the same protein molecule may contain two or more such groups. It may well be that the intact protein molecule is involved in the reaction (for there is but little evidence that anything less than an intact protein molecule is capable of producing the typical reaction), but that certain groups determine the specificity." (Wells and Osborne.[10])

Also, "since chemically similar proteins from seeds of different genera react anaphylactically with one another, while chemically dissimilar proteins from the same seed in many cases fail to do so, we must conclude that the specificity of the anaphylactic reaction depends upon the chemical structure of the protein molecule. Corroborative evidence has been furnished with precipitin reactions by Landsteiner and Lampl."[11] (Wells.)

Although the anaphylactic reaction is a less delicate means

[10] Wells and Osborne: Jour. Infect. Dis., 1913, xii, 341.
[11] Landsteiner and Lampl: Jour. Biochem., 1918, lxxxvi, 343.

of determining specificity than is the precipitin reaction, or the complement fixation method, the more general employment of the reaction in the isolated uterus strip has proved it to be equally sensitive. Thus Dale has been successful in differentiating crystallized albumins from hen's eggs and from duck's eggs. "In other words, a close relationship by immunological tests is here associated with chemical similarity, and a slight difference in chemical structure is found which presumably accounts for a slight immunologic difference that can be detected only by the most sensitive methods." (Wells.)

CHAPTER XIII

ANAPHYLACTOID PHENOMENA

The final explanation of anaphylaxis is still unsolved. Much work has already been carried out by numerous observers in an effort to throw light upon the subject. Since the primary parenteral introduction of certain protein substances is followed by manifestations of symptoms which simulate those which characterize the typical anaphylactic experiment, the question naturally arises as to the relationship of these substances to the anaphylactic antigen. In this chapter the salient features of the more important anaphylactoid phenomena are recorded and, in part, analyzed.

Endotoxins

Pfeiffer (1892) discovered that a given dose of cholera vibrios, which was harmless to a normal animal, sufficed to bring about rapid death of an animal which had previously been injected with a sublethal dose of vibrios. He used the intraperitoneal route of administration, and found that in the material removed from the peritoneal cavity, the microorganisms could no longer be identified as such. Lysis of the bacteria had taken place.

It was upon this experiment that Pfeiffer built his hypothesis of endotoxins. He believed that, as a result of the lytic activity of the body fluids in immune animals, the bacterial cell bodies were dissolved, and that a preformed toxic substance situated in the cell cytoplasm was liberated. This hypothetical poison he named endotoxin.

Experiments since that time tend to show that no such preformed toxic substance does, in fact, exist in bacterial cell bodies, but that, as the result of the interaction of the anaphylactic antibody (first order body) with the bacterial cell protein, there is produced an irritant substance. The exact na-

ture of the reaction which takes place between antibody and protein antigen is not, as is elsewhere shown, finally proved.

In 1902 Weichdardt[1] subjected rabbit's syncytial protein to the action of specific antisera and obtained substances which were toxic to normal rabbits. It is to be noted that this experiment was carried out before the discovery of the anaphylactic phenomenon. In 1904 Wolff-Eisner[2] formulated a theory which was essentially to the effect that specific cytolysis of bacterial and tissue cells resulted in the production of toxic substances. Wolff-Eisner[3] as a direct result of his experiments upon this subject proposed the employment of the conjunctival reactions to tuberculosis which was simultaneously described by Calmette[4] in France.

Anaphylatoxin (Friedberger)

Friedemann[5] found that if 3 c.c. of beef corpuscles were injected into rabbits, and the injection repeated after three weeks, anaphylaxis regularly occurred. Friedemann then treated sensitized red blood cells *in vitro* with alexin, and incubated the mixture He found that when the supernatant fluid was injected into normal rabbits, symptoms identical with those which characterize anaphylaxis supervened. In order to exclude any possible toxic action of hemoglobin the action of the alexin was arrested by cooling at a time just preceding the occurrence of hemolysis. Upon the basis of these experiments Friedemann expressed the opinion that anaphylaxis is due to the action of alexin upon sensitized protein antigen.

Friedberger[6] (1910) confirmed Friedemann's findings, and proved that the treatment of specific precipitates with fresh normal serum alexin was followed by the production of a toxic product. The following Friedberger experiment is quoted from Zinsser: "One cubic centimeter of a rabbit

[1]Weichdardt: Deutsch. med. Wchnschr., 1902, p. 624 (quoted by Zinsser).
[2]Wolff-Eisner: Centralbl. f. Bakteriol., 1904, xxxvii.
[3]Wolff-Eisner: Berl. klin. Wchnschr., 1907, p. 1052.
[4]Calmette: Compt. Rend. de l'Acad. des Sci., June, 1907.
[5]Friedemann: Ztschr. f. Immunol., 1909, iii.
[6]Friedberger: Berl. klin. Wchnschr., 32 and 42, 1910; also Ztschr. f. Immunol., 1910, iv.

serum which precipitated sheep serum in a dilution of 1 to 10,000 was mixed with 30 c.c. of a 1 to 50 sheep serum dilution. This was kept one hour at 37.5° C. and overnight in the night chest, when a heavy flocculent precipitate had formed. This precipitate was washed to remove all traces of serum, and to it was added 2 c.c. of fresh normal guinea pig serum—as complement. This was again allowed to stand for twelve hours and, then, the supernatant fluid was injected into a guinea pig intravenously. In most cases the pigs so treated showed marked symptoms soon after the injection and died within a few hours.''

It is evident that a specific substance is present in the serum of sensitized (and tolerant) animals which combines with or so acts upon certain specific foreign proteins that the product of the reaction is no longer soluble in an inactive serum mixture.

It is possible to dissolve out from the precipitate, by means of the addition of alexin, a substance which is soluble in a mixture of serum and salt solution, and which produces, upon intravenous injection into animals, symptoms of anaphylaxis. If inactivated guinea pig serum or sodium chloride be used in the experiment, the fluid is not rendered toxic.

As the result of these experiments, Friedberger formulated the ''anaphylatoxin'' theory of anaphylaxis. The essential characteristic of Friedberger's theory is that anaphylaxis is a true intoxication by a poison which results from the action of alexin upon the products of a precipitin-precipitinogen reaction. The poison so formed he designates by the term ''anaphylatoxin.''

One of the essential premises of Friedberger's hypothesis is that the anaphylatoxin is formed from the matrix of the protein antigen. This aspect of Friedberger's conception has been attacked by numerous writers (e.g., Weil and Zinsser).

Doerr and Russ proved that the precipitate which results from the mixture of immune serum and antigen may be dissolved in weak soda solution and that anaphylactic shock follows its intravenous injection.

Doerr and Russ have also demonstrated that the passive sensitizing property of serum is proportionate to its precipitating strength. This has been confirmed by Anderson and Frost, who further proved that the precipitate formed by the action of precipitating serum upon its antigen will cause anaphylactic shock in normal animals. They, therefore, assume that the anaphylactic antibody is identical with precipitin.

Similar results were obtained by Anderson and Frost.[7] Washed precipitate was treated with 1.8 c.c. of normal guinea pig serum and injected intravenously. Marked dyspnea appeared at once, the animal was quite sick for more than an hour. Anderson and Frost in 1910 remarked "immediate anaphylactic shock in normal guinea pigs, following the injection of mixtures of horse serum with specific antiserum, seems capable of explanation only by the assumption of an anaphylactic antibody. Whether the anaphylactic antibody acts as an intermediate link between antigen and body cells, or whether it serves to split up the serum into derivatives capable of direct combination, remains an open question."

Following experiments such as those just mentioned, the observations of Rosenow on the production of an anaphylactic substance by autolysis of bacteria, are of considerable interest. Rosenow found that injections of virulent pneumococci, at one stage of autolysis in salt solution, produced symptoms and pathologic changes characteristic of immediate anaphylaxis. In a subsequent paper he reports the repetition of experiments in which he employed streptococci, staphylococcus aureus, meningococci, gonococci, the colon bacillus, the bacillus pyocyaneous, the bacillus dysenteriae of Shiga, and the spirillum of Metchnikoff. Partially autolyzed suspensions of these bacteria yielded similar results. These results, states Rosenow, speak strongly in favor of the view that the symptoms in sensitized animals following a second injection of pneumococcus extract are due to the rapid splitting of the protein material, and that a similar splitting occurs at a much slower rate *in vitro* by autolysis.

[7]Anderson and Frost: Trans. Cong. Am. Phy. and Surg., 1910, viii, 429.

It is worthy of note that staphylococci were not subject to proteolysis unless treated with leucocytes or serum, although it is not necessary that either leucocytes or serum should be from an immune animal.

Protein Split Products (Vaughan)

One of the most valuable contributions to the subject of protein intoxication, and its relationship to immunity, is that of Vaughan[8] and his associates. Their chief conception, and the one upon which most of their experimental work has been brought to bear, is that every complex proteid—protein—consists of one common nitrogenous archon, or keystone, to which are attached a larger, or smaller, number of side chains. Each of the latter has a characteristic structure. It is to the variation in nature, and distributions of these side chains that proteins from different sources owe their differentiation from one another.

In accordance with Vaughan's conception, the keystone of the protein molecule is common to all proteins, and forms the primary group. Proteins differ from one another in their secondary and tertiary groups. Albumins and other complex proteins are not poisonous, because, in them, the chemism of the primary group is satisfied by combination with secondary groups. When the secondary groups are stripped off, the primary becomes poisonous on account of the avidity with which it combines with the secondary groups of other molecules.

By means of hydrolysis of the protein molecule, Vaughan believes that he has isolated, more or less crudely, the primary group. For this purpose he employed hot (78° C.) alkaline (2 per cent NaOH) alcohol. This primary group is referred to by Vaughan as the poison or poisonous protein split product. One milligram of protein suffices for the production of enough poison to kill a guinea pig.

When cleavage of the molecule is obtained by means of

[8]Vaughan: Protein Split Products and Their Relationship to Immunity and Disease, Lea & Febiger, 1918.

peptic digestion, the poison is formed about the stage of the formation of peptone. A similar poison was also obtained by Vaughan as a result of the action of blood serum, and organ extracts, from sensitized animals upon their specific antigens.

From all true proteins examined by Vaughan, a poisonous moiety has been isolated having identical properties, biologically considered, although in the form so far isolated, they are not chemically identical. This fact, in view of the observations of Wells and his associates, is, in all probability, due simply to difficulties in purification. The poisonous group in the molecule is not removed from its attachments to other groups by purely physical solvents. The molecule must be disrupted by high temperature, chemical agents, or enzymes, before this can be done.

When proteins are subjected to splitting in the manner described, there is obtained a soluble substance, which is the poison, and, also, an insoluble residue. When animals are injected with the soluble poison, symptoms identical with those which occur in typical anaphylactic shock are produced.

Repeated injection with the poison does not result in increased sensitiveness: nor does injection with the soluble poison, render the animal hypersensitive to the whole protein.

The insoluble residue is not toxic: it does, however, sensitize the animal against the unaltered protein. It is probable that this latter effect is due to the presence in the residue of a certain amount of protein, which thus induces hypersensitiveness to itself.

Vaughan believes that the splitting of the protein in the animal body is due to a proteolytic ferment, which is the product of certain cells. This ferment is specific for the protein, which calls it into existence. This in Vaughan's opinion is the explanation of the anaphylactic phenomenon.

Cleavage products can be produced, not only by hydrolysis by alcohol hydroxide solution, as employed by Vaughan, but also by means of a more prolonged treatment with acids or by exposure of the protein to high degrees of moist heat in

the autoclave. The substances procured by these methods differ, moreover, quantitatively only, from those produced by the ferment action of trypsin.

Animals which have been subjected to repeated sublethal injections of the poison (split product) are able to withstand from two to four times the amount of poison which is fatal in controls. Vaughan speaks of this protection as being due to tolerance, and not immunity. He makes no effort to explain the nature of tolerance, and states that more work is necessary upon the subject.

He also found that animals which had been treated with the poison derived from B. coli are more resistant than controls to the injection of living organisms,—at least two fatal doses may be tolerated. Guinea pigs treated with the residue showed acquired immunity to at least eight times the fatal dose of living organisms.

This protection, on the part of animals treated with the residue, I explain as being due to sensitization of the animal, with the result that upon subsequent injection of living organisms, a reaction occurs immediately after inoculation. The apparent immunity of such animals, is due to a condition of allergy.

White and Avery[9] have published the results of, and conclusions based upon, an exhaustive series of experiments dealing with the nature of the toxic alcohol-soluble moiety of tuberculoprotein after treatment with alcohol hydroxide. They found the minimal fatal dose of the product to be approximately 1 to 1500 parts of body weight. They employed, as experimental animals, guinea pigs, weighing 250 grams or more.

By means of the injection of the poison in suitable doses, and by various routes, it is possible to produce clinical manifestations of disease, including febrile reactions which simulate the various types of infectious disease.

"During the active progress of an infectious disease, the body cells supply the ferment, the injecting organism con-

[9]White and Avery: Jour. Med. Research, 1910, xxiii, 95.

stitutes the substrate, the process is essentially destructive, the protein poison is set free, the symptoms of disease appear and life is placed in jeopardy.'' (Vaughan.)

As pointed out by Zinsser[10] it is to be noted that Vaughan does not take for granted the formation of the poisons under the influence of the sensitizer-alexin mechanism in the circulation; and does not assume that the entire process necessarily takes place in the circulation.

In discussing the work of Vaughan and Wheeler[11] Zinsser makes the following remark: ''In its general significance, this work ranks among the most important contributions to our understanding of hypersusceptibility, though the theoretical deductions made from it have had to be subjected to considerable alteration.''

Peptone Poisoning

Biedl and Krause[12] have drawn a very close parallelism between anaphylactic shock and petone poisoning in dogs. They have shown that injections of peptone (0.3 gr. to the kilo) give rise to the same clinical symptoms that characterize anaphylaxis. It is accompanied also by typical fall of blood pressure, delayed coagulability of the blood, and leucopenia. These authors have also found that in guinea pigs, as well as in dogs, injections of peptone are followed by typical manifestations of anaphylaxis.

In guinea pigs, the symptoms of peptone poisoning are similar to those exhibited during anaphylactic shock. There is ruffling of the hair, respiratory distress and prostration. At autopsy the lungs are fully expanded and occasionally, subserous hemorrhages are observed. ''The marked resemblance between the gross and microscopic changes produced by injections of peptone and by injections of native proteins (in hypersensitive animals) furnishes another reason for considering these two phenomena closely related.'' (Boughton.[13])

[10]Zinsser: Jour. Immunol., 1920, v, 265.
[11]Vaughan and Wheeler: Jour. Infect. Dis., 1907, iv.
[12]Biedl and Krause: Wien. klin. Wchnschr., 1902, p. 11.
[13]Boughton: Jour. Immunol., 1919, iv, 381.

The irregular results obtained by different investigators regarding the toxic manifestations following peptone injection may be assumed to be due to the fact that commercial peptone (Witte) is simply a mixture of protein degradation products. Brieger[14] has found that those samples of Witte's peptone, which proved toxic, yield on extraction a substance which he had called "peptotoxin."

Whipple and Cook[15] found that proteose injections into fasting dogs are followed by a rise in nitrogen elimination to more than double the normal.

Artificial proteoses produced by tryptic digestion of egg white, as well as other more complete degradation products of hydrolysis, do not sensitize guinea pigs.

Heidenheim[16] has noted an increase in the lymph flow during peptone poisoning. As pointed out by Zinsser, this is of especial interest in view of a similar phenomenon noted by Calvary[17] in anaphylactic shock.

Histamine

Barger and Dale,[18] as the result of an enquiry into the nature and characteristics of Popeilski's vasodilatine (derived from intestine), discovered that boiled acid extracts of intestine contain the salt of a base which is also obtained by the splitting off of carbon dioxide from histamine, and is *B. imidazolethylamine.* Dale and Laidlaw[19] refer to the similarity of the reaction of this substance, which they call histamine, to that of the intoxicating agent in anaphylaxis, and have suggested that it may be the active principle concerned. Should the identity of these substances be proved, the value of the more recent experiments by Dale and Laidlaw[20] upon the reaction of the tissues to histamine, will be greatly enhanced.

Intravenous injection of 0.5 milligram of *B. imidazolethylamine or histamine* into large guinea pigs, results in respira-

[14] Brieger: Die Ptomaine.
[15] Whipple and Cook: Jour. Exper. Med., 1917, xxv, 461.
[16] Heidenheim: Pflüger's Arch., No. 49, 1891. (Quoted by Zinsser.)
[17] Calvary: Münch. med. Wchnschr., 1911, xiii.
[18] Barger and Dale: Jour. Physiol., 1910, li, 499.
[19] Dale and Laidlaw: Jour. Physiol., 1910, li, 318.
[20] Dale and Laidlaw: Med. Res. Com. Mem., on Shock, February, 1917.

tory difficulties, convulsions and death. At autopsy distention of the lungs, typical of anaphylactic shock, is found. Treatment of the animal with atropin diminished the severity of the reaction, just as Auer and Lewis[21] found this to be the case in true anaphylaxis. In dogs, fall in blood pressure characterizes the reaction. It would seem then that substances representing cleavage of native proteins of highly complex nature, the result of proteolytic cleavage not very far advanced, are probably concerned in the production of anaphylactic shock.

Histamine has a synergetic relation to anaphylactic shock (M. I. Smith[22]). This may be either because the point of contact to histamine and the anaphylactic irritations are the same, or because they are closely related to one another.

The chief respects in which histamine fails to account for all the phenomena of anaphylaxis are, according to Wells:[23]

1. It fails to desensitize animals or tissues, yet produces strong reactions in the uterus strip that has been thoroughly desensitized. (Dale.[24])

2. Histamine does not produce the temperature reactions usual in anaphylaxis.

3. Histamine does not produce the changes in coagulability of the blood usual in anaphylaxis.

4. Quinine augments the susceptibility of sensitized animals to anaphylactic shock, but not to histamine poisoning.

There is no reason for expecting that histamine, even though it be identical with the anaphylactic poison, should desensitize anaphylactic animals or tissues. The antibody which is exhausted during desensitization is the substance which is responsible for the production of an irritant through its reaction with antigen. Up to the present no experiments have been attempted which would indicate that repeated sublethal injections of histamine induce tolerance, nor that tolerance is exhausted by histamine introduction.

Since the effect upon temperature in anaphylactic experi-

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[21]Auer and Lewis: Jour. Am. Med. Assn., 1909, liii, 458.
[22]Smith, M. I.: Jour. Immunol., 1920, v, 239.
[23]Wells: Physiological Review, 1921, i, 44.
[24]Dale: Jour. Pharmacol. and Exper. Therap., 1913, iv, 167.

ments is dependent largely upon the dose of antigen employed and the route of administration, the fact that typical temperature changes have not been observed in histamine poisoning cannot be accepted as final proof that histamine shock and anaphylaxis are not related to one another.

With regard to the fact that quinine augments the susceptibility of sensitized animals to the antigen it is altogether likely, as Wells points out, that it is the antigen-antibody reaction which is aided, and not an increased sensitiveness on the part of the tissue cells to the product of this reaction.

Not only does histamine poisoning mimic anaphylactic shock in the guinea pig and in the dog, but also in the rabbit. Dale and Laidlaw[25] have noted that the cause of death in histamine poisoning was pulmonary obstruction which leads to acute dilatation of the right heart. This in Coca's[26] opinion is the cause of death in anaphylaxis in these animals.

Taraxy (Novy). "Nonspecific Anaphylaxis"

Experiments by Novy and DeKruif,[27] published in 1917, show that a type of shock indistinguishable, either clinically or at autopsy, from specific anaphylaxis may be produced by the addition of serum *in vitro* by a variety of substances, agar, peptone, inulin, kaolin, distilled water, organ extracts, and numerous other substances usually looked upon as nontoxic. Similar phenomena follow intravenous injection of suitable amounts of these materials.

These observers suggest the hypothesis that in true specific anaphylaxis and "peptone anaphylaxis," as well as in the type of nonspecific anaphylaxis which they have investigated, the matrix of the poison is not the antigen introduced, but is a normal constituent of the tissues (blood). The foreign substance which induces the formation of the toxic body, whether these foreign substances be the result of the specific interaction of some newly formed substance in the sensitized animal with the injected protein, or whether it be represented by the agar or distilled water of their experiments, acts as a catalyser

[25] Dale and Laidlaw: Jour. Physiol., 1911, xliii, 182.
[26] Coca: Jour. Immunol., 1919, iv, 219.
[27] Novy and DeKruif: Jour. Am. Med. Assn., 1917, lxvii, 1528.

in a manner similar to that of the inducing substance in the formation of fibrin from fibrinogen. These authors found a very important factor in the physical state of the agar, and that ferment action is probably not all it involved.

Novy and DeKruif also found that occasionally transfusion of blood, from animals in which nonspecific anaphylactic shock had been produced, was followed by manifestations of toxic effects in recipient guinea pigs.

Anaphylactoid Phenomena Due to Flocculation of Colloids

A most interesting series of experiments have been carried out by Karsner and Hanzlik, and by Kopacrewski. These two groups of observers differ in their explanation of the phenomena which they have encountered, and in their relationship to the anaphylactic reaction. Karsner and Hanzlik have shown that agar and numerous other substances produce anaphylactoid symptoms after intravenous injection. The symptoms, they believe, occur as a result of occlusion of the pulmonary capillaries. This is due to thrombosis or to agglutination of corpuscles or platelets. The capillary obstruction is demonstrated both by the identification of emboli, and by arrest of perfusion fluids injected into the pulmonary vessels. In consequence of this capillary obstruction, there may occur a marked bronchial spasm which causes asphyxia and permanent obstruction of the lungs. They state, "On the basis of the results obtained with atropin and epinephrin, the mechanism of the action of agar and similar agents bears no relationship to anaphylaxis or anaphlyactic shock."

Concentrations of agar as low as 0.001 per cent cause agglutination *in vitro:* other colloids act in varying concentrations (e.g., acacia, 1.0; althea, 0.08; collargol, 0.005; gelatin, 0.05; nuclein, 0.5; beef serum, 0.005 per cent). "The quantity of agar necessary to produce profound effects, or even death, is remarkably small, namely, about 0.014 to 0.05 mg. per gram of body weight."

In order to avoid confusion as the result of such occlusion of pulmonary capillaries, Wells has usually selected the intra-

peritoneal route for the performance of anaphylactic relations. For the same reason, Besredka has since 1907 employed the intracerebral method of injection of the exciting dose.

Kopacrewski[28] points out that widely diverse substances which have proved useful in the prevention and amelioration of anaphylactic shock all have one or two traits in common. All such substances either prevent flaking in the blood serum, or they dilate the blood vessels. Kopacrewski believes that his experiments prove that anaphylactic shock is the result of physical changes in the blood serum which permit flocculation of the molecules. Dark ground illumination has shown that flaking occurs in blood serum, when the latter is brought in contact with certain colloids. The surface tension of the serum of animals that have died from anaphylactic shock, he has found to be much reduced. This is a phenomenon which always accompanies colloidal flocculation. Kopacrewski is of the opinion that capillary embolism in the lungs is characteristic of anaphylaxis, and that it is due to the flocculation. His explanation is that the introduction into the serum of a normal animal of one of a group of colloidal substances upsets the colloidal balance, and there is flocculation of the molecules. The flakes, thus formed, obstruct the capillary network, and thus induce explosive asphyxia. Prophylaxis and treatment demand the administration of substances that reduce the superficial tension of the blood serum (saponia, soaps, bile salts, anesthetics, hypnotics, lecithin, etc.), or to render the serum more viscous (sugars, glycerin, acacia, carbonates, alkalines, etc.) or to dilate the blood vessels and thus allow the passage of the flaked micella (calcium lactate, atropin, etc.). No facts have been so far discovered, he declares, which conflict with this physical conception of anaphylaxis and anti-anaphylaxis. Kopacrewski injected into the blood of animals an amount of anesthetic which was equal to that found in the blood of an anesthetized animal, although the animal did not show signs of anesthesia. This animal was protected against anaphylactic shock in the same way as the anesthetized animal.

[28]Kopacrewski: Ann. de Med., 1920, viii, 291.

CHAPTER XIV

SITE OF THE REACTION BETWEEN ANTIGEN AND ANAPHYLACTIC ANTIBODY

With regard to the process of development, origin or site of the production of the anaphylactic toxic body, there are at the present time several theories, each of which is insufficiently supported by experimental evidence. Upon one subject, only, is there unanimity of opinion, namely, that the irritant substance, responsible for the manifestations of anaphylaxis, is the result of an interaction between a specific antibody (anaphylactic—first order antibody), which is produced by the animal tissues under stimulation, and the injected protein antigen.

In "active" anaphylaxis the antibodies are present as the result of the reaction to a preceding antigen injection. In the "passive" condition they were conveyed with the injected antiserum (Zinsser).

Two theories, in particular, have been advanced and much experimental data published in order to support each of the two views, respectively.

The first of these theories, and one which obtained a keen and able protagonist on this Continent in the person of the late Richard Weil,[1] assumes that the sensitive animal is such because certain of its tissue cells have produced sessile receptors, which have an affinity for the specific antigen: and that the protection from injurious action enjoyed by immune (tolerant) animals is due to the fact that the blood plasma of immune animals contains free antibodies so that the fixed receptors, attached to the cells, are guarded against the action of the injected protein. This hypothesis appears to assume that proteins, as such, are toxic to such cells as possess recep-

[1]Weil: Jour. Med. Research, 1913, xxvii, 497.

tors capable of fixing them, and that the hypersensitive, or anaphylactic, state is developed as the result of the production, on the part of the cells, under stimulation, of such fixing receptors. The further development of free receptors as the result of repeated sublethal injection, according to this point of view, results in the protection of the cells through an absorption of the toxic protein by such circulating receptors.

This theory is simple and is in accord with Ehrlich's hypothesis. It is, too, supported by a certain amount of experimental evidence. There are, however, certain established facts relative to the nature of anaphylactic toxicity which are entirely disregarded by the supporters of this theory; which facts, moreover, are quite sufficient, in my opinion, to render such an hypothesis untenable.

The fundamental differences between the so-called humoral and the cellular theory are as follows: According to the cellular theory, it is assumed that only such antibodies as are fixed to, and have become integral parts of, tissue cells, are able to react with injected antigen in such a way as to injure the tissue cells. Antibodies free in the body fluid do not take part in any reaction which results in the production of an irritant.

According to the so-called humoral point of view the reaction between antigen and antibody results in the development of a tissue irritant. This latter substance causes injury to, or at least induces reaction in, such cells as are susceptible to its action.

Those investigators (Weil,[2] Besredka[3]) who most earnestly support the conception of anaphylaxis and immunity as consisting simply in the primary production of "sessile receptors" and ultimately of free "immune bodies," lay great stress upon the fact that according to their theory, immune processes are shown to be cellular and that any other point of view assumes that the process is entirely humoral. This attitude is, in the author's opinion, unreasonable, since it is obvi-

[2]Weil: Jour. Med. Research, 1913, xxvii, 497.

[3]Besredka: Compt. Rend. de la Soc. de Biol., 1907, lxiii, 294.

Besredka-Gloyne: Anaphylaxis and Anti-anaphylaxis, 1919, C. V. Mosby Co., St. Louis.

ous that whatever may be the correct explanation of these phenomena, the process must be due to cellular activity and ultimately to cellular irritation.

Obviously all antibodies present in the body fluids must primarily have had their origin in tissue cells. Presumably also, it is only such cells as normally have the property of combining with foreign molecules that can be expected to produce such antibodies. Consequently, it is not surprising that at an early stage in the development of the anaphylactic state the antibodies responsible for the reaction are intimately associated with those tissue cells which are responsible for their production, and that, toward the period in which hypersensitiveness to the protein antigen is lost, only in those cells, which were primarily responsible for the production of the antibodies, will antibodies be present.

According to the hypothesis of cellular sensitization, hypersensitiveness of the tissue occurs as the result of the development on the part of certain of the tissue cells of specific affinities or receptors. By reason of these acquired affinities, these cells fix or anchor the injected protein and thus accomplish their own injury. It would appear that according to this theory all foreign proteins must be assumed to possess injurious properties for all cells able to anchor them. It must be further assumed that even though different protein molecules differ from one another in chemical arrangement they are all capable of affecting sensitized cells in a like injurious manner, since, in the same species of animal, the same symptoms of intoxication follow the injection of a large number of proteins from different sources.

The so-called humoral theory, or the theory of antigen-antibody reaction, conceives the changes which occur in the animal body during the process of sensitization to be as follows: Whereas the majority of complex proteins—albumins and globulins—are relatively innocuous when parenterally introduced into the tissues of normal animals, such injections are followed by the production, by certain tissue cells, of antibodies which so react with subsequently introduced anti-

gen that a substance, which acts as an irritant to the tissue cells, is produced.

The basis upon which these two hypotheses are termed cellular and humoral, is derived from the result of transferred anaphylaxis. Exponents of the "cellular" theory maintain that the injected animal is rendered anaphylactic by reason of the withdrawal of the free receptors present in the serum of repeatedly inoculated animals from the circulating blood, and their fixation by the tissue cells. The so-called "humoralists" explain the occurrence of transferred or passive anaphylaxis by simply stating that the injected animal receives the protein altering (splitting) substance produced by the tissues of the actively treated animal, and that, as a result, the specific antigenic protein, when subsequently introduced, is rapidly altered with the production of irritant substances (toxic split proteins).

The chief stumbling block, interfering with the general acceptance of the theory of antigen-antibody reaction as opposed to cellular sensitization, is that which arises from the fact that passive transfer of the anaphylactic state appears to require the lapse of a definite period of time between injection of the sensitizing serum and its antigen. Passive sensitization of normal animals is produced with great regularity if an interval—incubation period—is permitted to elapse between the introduction of the sensitizing serum and the intoxicating injection On the other hand, it has been found difficult to induce anaphylaxis if the transferred serum and the antigenic protein are introduced immediately following one another. It is during the incubation period that the sessile receptors of the donor's serum are supposed to become fixed by the tissue cells of the recipient; thus, upon the basis of cellular hypothesis, this phenomenon (incubation period) is easily explained.

According to the theory of antigen-antibody (humoral) reaction no such period of incubation should be necessary, although it does not seem unreasonable to suppose that the more intimately the injected antibodies are brought in con-

tact with such cells as are susceptible to irritation by the product of antigen-antibody reaction, the more marked will be the manifestations of intoxication on the part of these cells.

The question as to whether, in order that an animal may become hypersensitive, the injected anaphylactic antibodies must first become an integral part of the tissue cells, is of fundamental importance. Experiments performed by the author and published in 1914, as well as those by Doerr and Russ, have proved that, although more reliable results are obtained in performing experiments of transferred anaphylaxis, if an incubation period be allowed to elapse, such an incubation period is not essential. It is possible both by the simultaneous injection of sensitizing serum and antigen, and by the injection of mixtures of sensitizing serum and antigen, to induce immediate manifestations of anaphylactic shock.

The "humoral" conception of anaphylaxis and immunity is based in part upon analogy, and in part upon direct experiment. The majority of native proteins are not poisonous to normal animals. It is now well established that the serum of sensitive and immune (tolerant) animals contains substances which are capable of inducing alteration of the protein molecule with the result that a highly toxic substance is formed or liberated. Degradation products of protein cleavage closely related to the peptones and proteoses, at least insofar as this relationship refers to the stage in the digestive process at which they are produced, are potent to produce, upon intravenous injection into animals, symptoms which are indistinguishable from those which characterize anaphylactic shock.

The humoral theory assumes the hypersensitive state to be due to the presence in the tissues of the animal, of antibodies which react with the injected protein antigen in such a way that a product is formed which directly or indirectly induces manifestations of irritation on the part of the tissue cells.

That these antibodies must be produced by the cells of the body is physiologically obvious, and that they should persist in close association with the cells responsible for their production so that animals remain sensitive even after demon-

strable amounts are absent in the serum, seems, *a priori* at least, equally reasonable. That, moreover, following the passive sensitization of normal animals the antibodies injected should become localized in certain cells does not appear to be entirely unlikely. Such facts, therefore, as the difficulty of producing transferred sensitization without the lapse of a few hours following the introduction of the sensitizing serum and the diminution—as proved by Weil and others—of the amount of circulating anaphylactic body in the serum, do not in any way disprove the adequacy of this hypothesis.

By, perhaps, the majority of recent observers and students (Weil, Schultz, Zinsser) of the subject of anaphylaxis the fact that, in passive sensitization of guinea pigs, more reliable results are obtained if an incubation period, of at least four hours following the injection of the sensitizing serum, is allowed to intervene before testing for hypersensitiveness, has been accepted as proof that the sensitizing antibody must be attached to suitable cells before anaphylaxis can be demonstrated. This view is opposed fundamentally to that championed by Friedemann and Friedberger, namely; that the irritant substance is a result of a reaction between soluble circulating antibody and its specific antigen.

Other important experiments, which appear at first sight to invalidate the humoral hypothesis, are those of Schultz. This author showed that isolated segments of intestine or uterus from sensitized animals react to the specific antigen by contraction. Schultz' observations have been further developed by Dale and by the late Richard Weil. There are two explanations, each of which in part explains both the effect of the incubation interval and Schultz' experiments. On the one hand it is evident that, granted a given minimal amount of sensitizing antibody, its effect upon sensitive cells will be more evident, if that sensitizing body is present in the body fluids, bathing the cells or within the cells themselves.

That the reaction between anaphylactin (first order antibody) and antigen should be less effective if it takes place within the circulating blood, than if it is in closer association

with the body cells, is to be expected. That it is necessary for the anaphylactic antibody to become an integral part of the cell in a chemical sense is, in the author's opinion, highly improbable. A more adequate conception of the nature of the reaction is obtained, in my opinion, if the animal body be looked upon as consisting of fluid in which the various cellular elements are suspended, and with which they are saturated.

It may well be that the following is an explanation of the apparent fact that the unstriped muscle tissues of the body absorb proportionately more of the injected anaphylactin than do other tissues. There can be but little doubt that, to use the terminology of Ehrlich, the unstriped muscle tissues have receptors which make it possible for them to anchor circulating heterologous proteins from the body fluids with more avidity than other tissues. If this be the case, it is to be expected that when a foreign serum, e.g., rabbit serum, containing anaphylactic antibodies is injected into a guinea pig, more of the injected serum will be absorbed by the nonstriated muscle cells as in the bronchial walls, the hepatic vessels, and the uterus, than by other tissues. One of the natural results of such a determination of the injected antigen would be saturation of these cells by the antibody constituent of the injected serum to a greater degree than other cells of the body.

Another possible explanation for the incubation period, and the one which is doubtless active when large doses of "immune" serum are used in transferred sensitization, is that both sensitizing and tolerant bodies are present in such sera, and that unless the relative proportions of sensitizing serum and antigen are exactly suitable in the dilutions, which exist in the circulating fluid, no manifestations of irritation are exhibited, until such time as, through a process of diffusion, the relative potency of the tolerant body has been diminished.

The fact that isolated guinea pig uterus segments, even though thoroughly washed by perfusion experiments, still react to the presence, in the fluid in which they are suspended, of the specific antigen to which the animal, from which the

uterus was removed, had been sensitized, may be explained as being due to the fact that the individual cells are themselves filled with body fluid. It is obvious that it is impossible to prove this point by direct experiment since it would be argued that, if sufficient washing of the uterine segments were persisted in, to remove the intracellular body fluid, failure of the muscle to react would be interpreted as being due to death of the cell.

Pearce and Eisenbrey report experiments in which, as a result of transfusion of the blood of a hypersensitive to a normal animal, and from a normal to a hypersensitive animal, they attempted to induce anaphylactic shock in both animals. They found that when both the animals, each of which weighed about 7,000 grams, were injected with 5 c.c. of horse serum, the hypersensitive animal, which had had its blood replaced by transfusion from a normal animal, reacted immediately by a fall in blood pressure from 90 mm. to 24 mm. The normal animal, which had received blood from the hypersensitive dog, which had been treated by 5 c.c. of horse serum subcutaneously twenty-five days previously, showed no change in pressure except slight mechanical rise.

These experiments are interpreted by their authors as proving that the reaction between antigen and antibody occurs only when the latter are fixed to the cells. It must be pointed out, however, that this is not necessarily the case, since in an animal sensitized, as was dog B, with but one injection of horse serum, very few circulating antibodies are ordinarily present. The absence of reaction, therefore, in the recipient of such blood is of relatively little importance. On the other hand, although the hypersensitive animal had its circulating blood fluid removed and replaced by normal blood, the proportion of body fluid remaining in the tissues was subjected to relatively little diminution.

Experiments by Doerr and Pick, which show that, even after all demonstrable antibodies have disappeared from the circulating blood in the rabbit, fatal anaphylactic shock may

be produced, as pointed out by Wells, may be employed as proof of the humoral as well as for the cellular hypothesis.

The evidence at present available indicates that the antigen-antibody reaction, responsible for the phenomenon of anaphylaxis, takes place wherever these substances are brought in contact with one another. If the reaction occurs within the cytoplasm of susceptible cells, the manifestations of tissue irritation is more marked than if a similar reaction occurs in the circulating blood or in body fluid removed from the site of cells susceptible to the product of the antigen-antibody reaction.

Wells[4] objects to those experiments which have been reported that when suitable quantities of antigen and antibody are used it is sometimes possible to secure evidence of passive sensitization without the usual period of incubation, on the ground that the results are obtained only occasionally, are usually slight in character, and without the necessary regularity to be convincing proof of a fundamental principle. The author has attempted to show what may be the reason that regularity in reactions of this sort is difficult to obtain. Weil earnestly (see page 164) doubted the true anaphylactic character of those reactions which have been observed. In my experiments the results obtained were typical of those ordinarily characterizing anaphylactic shock in the guinea pig.

In discussing the source of the toxic substance in the anaphylactic reaction Wells states: "The 'anaphylatoxin' hypothesis fitted most of the known facts so well, and was so perfectly logical, that it seemed almost inevitable, yet at the present time it appears to be untenable, in the face of existing evidence, as the final explanation of anaphylaxis." The demonstration that from almost any protein a toxic moiety can be produced by cleavage which produced effects in animals, closely resembling anaphylaxis, and by Friedberger that mixtures *in vitro* of antigen-antibody and complement become highly poisonous as well as the similarity of "peptone" and anaphylactic shock, is at least suggestive that anaphylaxis is the result of

[4]Wells: Physiological Reviews, 1921, i, No. 1.

a toxic substance resulting from proteolysis induced by the action of alexin, after sensitization of the antigen by the anaphylactic antibody. (Wells.)

"On the other hand, there is no doubt that antigen-antibody reactions do produce, at least *in vitro,* substances that are eminently injurious, especially on intravascular injection, and it seems reasonable indeed to believe that such substances may be produced in the typical anaphylaxis reaction and play some part in it, even if we grant that the typical anaphylactic shock depends on reaction within certain tissue cells. For example, Manwaring and Kusama,[5] found that although isolated lungs from sensitized guinea pigs exhibited strong bronchial constriction when perfused with the specific antigen, they gave still stronger reactions when the blood of the sensitized animal was present in the perfusion fluid (Wells)."

Wells sums up the points that have been advanced against the so-called humoral anaphylatoxin theory as follows: Possible explanations of these objections are also quoted from Wells:

1. "It does not fit with the latent period of passive sensitization. However, intracellular formation of anaphylatoxin might account for this phenomenon." As the author has elsewhere shown, this incubation period is not an essential characteristic of passive sensitization. Theoretically, the more reliable results which are obtained if a time interval be permitted to elapse can be explained.

2. "Complement is not essential, since animals deprived of free complement in the circulating blood may still give anaphylactic reactions. Here again, one may suggest the presence of intracellular or reserve complement." It should be pointed out, moreover, that it is doubtful whether this statement is, in fact, correct.

3. "All attempts to prove that complement is a proteolytic ferment have so far failed." Nevertheless, numerous experiments suggest that this is the case.

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[5]Manwaring and Kusama: Jour. Immunol., 1917, ii, 157.

4. "Anaphylatoxin" activity has been produced in serum by digestion in the absence of complement, in the absence of antigen, and in the absence of antibody. On the other hand if antigen and the specific antibody are injected simultaneously into the opposite jugular veins of a guinea pig, the animal shows no evidence of intoxication." In a separate section the fact that although the mechanism of "anaphylatoxin" production by more specific methods may differ from that of ordinary anaphylaxis, the result may well be identical, namely—degradation of protein with liberation of its toxic moiety. The author's experiments disprove the assertion contained in the second sentence of this paragraph.

5. "In the anaphylatoxin experiments the existence of capillary embolism or endothelial intoxication has not been excluded, and there is reason to believe that most of the observed symptoms are anaphylactoid rather than anaphylactic."

6. "All attempts to demonstrate that the blood of animals in anaphylactic shock contains a poison responsible for the observed manifestations, have failed (see Weil[6])." It is difficult to imagine that, even though it be assumed that a substance of the nature of "anaphylatoxin" be produced in the body fluid, the toxic substance should remain in the circulating blood long enough to permit of its identification.

It will be seen that, although Wells allies himself, in general, with those who are opposed to the humoral anaphylatoxin theory, he considers the question as not definitely decided. He writes:

"But we cannot escape the fact that the manifestations of anaphylactic shock resemble in all respects those of an acute intoxication: furthermore, that histamine, the substance which produces the picture most closely resembling that of typical anaphylaxis, is a product of protein cleavage (see Abel and Kubota,[7] Dale,[8] Hanke and Koessler[9]). Not only does histamine cause bronchial spasm in guinea pigs, obstruction to

[6]Weil: Jour. Immunol., 1917, ii, 399.
[7]Abel and Kubota: Jour. Pharmacol. and Exper. Therap., 1913, iv, 167.
[8]Dale: Bull. Johns Hopkins Hosp., 1920, xxxi, 257, 310.
[9]Hanke and Koessler: Jour. Biol. Chem., 1920, xliii, 521-579.

pulmonary circulation in rabbits and fall of blood pressure in dogs, but applied to the skin or mucous membranes, it causes marked local urticaria resembling closely that of skin reactions in sensitized persons, and it does all these things in extremely minute dosage, comparable with the dosage of proteins used in the anaphylactic reaction. Furthermore, its antecedent amino acid, histidine, is present in every known complete protein. Other pure chemical products of protein cleavage, such as methyl guanidine, have more or less similar effects."

CHAPTER XV

NATURE OF THE REACTION BETWEEN ANAPHYLACTIC (FIRST ORDER) ANTIBODY AND ANTIGEN

In 1910 Rosenau and Anderson presented the following view of anaphylaxis in the guinea pig:

Hypersusceptibility to a foreign protein consists in an increase in the normal power of assimilating this protein, especially an increase in the rapidity of the reaction. This is due to the formation of a specific antibody (or antibodies), demonstrable both in somatic tissues (smooth muscle) and in the serum of sensitive guinea pigs. The action of this antibody upon its antigen is quantitative, and probably primarily proteolytic. The action of this antibody apparently needs to be aided by the nonspecific alexin of the blood. The structure of the antibody concerned is, of course, unknown, as is also the nature of the chemical changes produced by it.

Rosenau and Anderson believed anaphylactic shock to be a disturbance of metabolic equilibrium due to temporary deflection of the normal metabolic activity of the tissue cells, rather than the result of any specific toxic action.

By certain observers, notably von Pirquet and Shick, it is thought that tissue irritation is the result of a reaction between two bodies (allergin and antigen) with the formation of a toxic substance. Richet, too, believes this to be the case, and terms the antibody present in the serum, toxogenin; the end product he designates by the expression apotoxin. Another series of observers, in particular Pfeiffer, Weichardt and Wolff-Eisner, regard the toxin as being preformed in the protein molecule, and to be merely liberated by the action of the antibody. According to this hypothesis, each bacterium or other protein substance contains a specific endotoxin.

Whether the product of the reaction between first order antibody and antigen is a poison in the ordinary sense, or

whether it represents a process which causes a discharge of energy after the nature of an electrical stimulus, is not known. That the explanation of the reaction between the antigen-antibody reaction product and the affected cell is to be found in an alteration in the colloidal state of the molecules constituting the latter, is not unlikely. That it acts as a tissue irritant is obvious. In the following pages are discussed the more probable methods whereby this tissue irritant may be developed.

It has been recognized for a number of years that immunity treats of the adaptation of the body tissues to the presence of foreign proteins.[1] Prolongation of life of the individual depends, in part, upon the ability of the organism, or certain specially differentiated parts thereof, so to act upon and alter complex protein molecules that they may be rendered useful, or at least not injurious to the individual's component cells.

Native proteins in the circulation or body fluids of the higher animals are not only useless, but are often directly harmful to the tissue cells. Phylogenetically it might be expected, and practically it may be proved, that the tissue cells are by no means helpless to protect themselves against such deleterious influences, for it has been proved, particularly by Alberhalden and his associates, that, within twenty-four hours after the injection of foreign proteins, digestive enzymes may be identified in the serum; although such ferments are not present in demonstrable amounts in the normal, noninjected animal. Direct evidence of the digestive activity of the substances produced by the body tissues under such circumstances has been obtained by Abderhalden by means of the polariscope and by dialysis experiments.

Vaughan conceives the poison isolated by him to be the keystone or archon of the protein molecule. It is the primary atomic group and is common to all protein molecules. One protein differs from another in the secondary and tertiary groups. Ordinary proteins are not poisonous because

[1]The problems of immunity narrow themselves down to special problems bearing upon the (parenteral) digestion and assimilation of unusual protein matter, or at least of the primary products of cell metabolism (Adami).

in them the chemism of the primary group is satisfied by combination with secondary groups. When the secondary groups are stripped off by hydrolysis or ferment action, the primary group becomes poisonous on account of the avidity with which it combines with the secondary groups of other molecules. Injury to the cells, of which the latter molecules form a part, is thus accomplished. The specific serologic reactions which are provoked by protein antigens are due to the secondary groups of their molecules.

In the author's opinion, the data at present at our disposal justifies the adoption of the following hypothesis of the immunologic process.

Large molecular protein complexes cannot be made use of by the tissues and are recognized as foreign bodies by the tissue cells. In consequence, certain cells produce substances which are known as antibodies (first order), whose function it is to bring about cleavage or disintegration of the complex protein. This is the first step in the immunologic reaction to the presence in the tissues of foreign proteins. Complete cleavage of the protein molecule to the stage of amino acid formation renders the protein not only harmless, but subject to utilization by the tissue cells. This stage of complete protein degradation is not, however, accomplished by the first order of antibodies, which are potent only to cause partial cleavage. Partial protein degradation products (to the stage of peptone-proteose formation) cause symptoms of tissue irritation.

When the tissues contain a considerable proportion of antibody (first order) which is capable of disintegrating protein to the irritant stage, the animal is said to be hypersensitive to the parenteral introduction of the whole protein. At this time the rapid introduction of antigen into the blood stream, or by any other route which permits of rapid absorption of even small quantities of the whole protein, provokes anaphylactic shock. When susceptible to anaphylactic shock in this way, the animal is said to be in the anaphylactic state.

Repeated introduction of a protein into the tissues stimulate

the production of a second order of antibody, whose function is the more complete dissociation of the protein molecule. When this second order antibody is present in the tissues, the latter are not susceptible to injury consequent upon the parenteral introduction of moderate quantities of antigenic protein. When in this state, the animal is said to be tolerant.

The Theory of Parenteral Digestion

Although several links in the chain of evidence, proving that the reaction which takes place between anaphylactic antibody and specific antigenic protein consists in a cleavage of the latter, remain to be forged, a reasonable amount of data upon which to base such an hypothesis has already been accumulated.

Briefly recapitulated, these data comprise the following observations: Cleavage products of complex proteins produced by the action of heat or chemicals are potent to induce symptoms of intoxication in normal animals, which are indistinguishable from those which occur in anaphylactic shock (Vaughan). Peptone and proteose mixtures produced by the digestive action of animal ferments possess similar properties (Biedl and Kraus). If immune sera and antigenic protein be mixed, in suitable proportions, and incubated, a highly toxic substance is produced (Friedemann). Specific precipitates when dissolved in an excess of fresh normal serum demonstrate like properties (Friedberger). The author's experiments in the production of anaphylactic shock, by means of the simultaneous injection of antigen and antibody, show that a similarly irritant substance may be produced in the body of normal animals.

It has been proved that anaphylaxis is a phenomenon, which occurs as the result of the repeated introduction of foreign protein substances parenterally into the tissues of an animal. Experiments suggest that it is possible that digestive processes may take place in the tissues of animals, analogous to those which occur normally in the alimentary tract.

One of the chief natural foodstuffs of the animal organism

consists of albumin in one or other form. These proteins are ingested as meats and various sorts of vegetable products and are digested in the alimentary tract. Subsequently, they are absorbed into the lymphatic and blood streams where they are available for the use of the tissue cells. The process of digestion of proteins consists essentially in hydrolysis, whereby the more complex albumin or globulin molecule is broken down to form first, peptones and histones, and ultimately polypeptids and amino acids, such as tryptophan, zein, cystine, etc. These amino acids, it has been proved, may be injected with impunity into the tissues, in which event not only are they innocuous, but they are subject to utilization by the body cells.

Not so, however, the cleavage products which appear at the stage of peptone formation, which have been proved by Biedl and Kraus, and others, to be capable of inducing reactions similar to anaphylactic phenomena when injected into normal animals. It has been shown by several observers, employing different methods, that, during the process of proteoclasis—by ferments, heat and chemicals,—there are developed degradation products of the protein molecule, which are extremely toxic. (Biedl and Kraus,[2] Vaughan, Jobling, Friedberger, Rosenow, Dick.)

These split products are developed at the stage in hydrolysis which corresponds to that at which peptones and proteoses are produced. The symptoms provoked by such split products correspond closely to those which occur during anaphylactic shock. Such being the case, and since similar phenomena are produced following the injection of the products of the interaction of serum from immune animals and specific antigenic protein (Friedberger, Friedemann), it is believed by many observers that the phenomenon of anaphylaxis is a concomitant of the exhibition of parenteral digestion.

When the sensitized or immunized animal is injected with a specific antigen, an increase of protein degradation products may be demonstrated both in the blood and in the urine. The

[2]Biedl and Kraus: Wien. klin. Wchnschr., 1909, No. 11, p. 363.

recognition of these facts was in part responsible for the development of the theory that protein antigen, when submitted to the action of alexin after it has been sensitized by the specific antibody (first order) is broken down or digested. This process, when it occurs in the body-tissues, is called "parenteral digestion." As has been noted, certain of the degradation products of proteins, notably peptone, produce all the symptoms of anaphylaxis, when injected into dogs. The conception, therefore, originated that parenteral digestion of foreign proteins gives rise to intermediate products of protein degradation which are responsible for anaphylactic shock, and for the symptoms of infectious diseases. Friedberger gave these products the collective name of anaphylatoxins; Vaughan identifies them with the toxic moiety which he has developed by means of hydrolysis of proteins.

Reasoning by analogy from other immunity experiments, and from the processes of intracellular digestion as carried out in the unicellulae, such as the amebae, it seems not unlikely that although certain tissues, notably those of the alimentary tract, are especially differentiated for the purpose of producing digestive substances, this same property of breaking down proteins is maintained, to a greater or less degree, by certain of the fixed tissue cells of the higher animals.

Support is given to the conception of parenteral proteolytic digestion by the experiments of Abderhalden. This investigator has been able to show by means of the refraction index of protein molecules, as determined by the polariscope, and by dialysis experiments, that the interaction of serum antigen and serum from a sensitized animal is followed by a breaking up of a certain proportion of the protein content of the serum antigen into simpler bodies. Dick has corroborated these findings, employing in place of serum antigen, protein material derived from the pneumococcus.

In this process of protein cleavage highly active toxic substances are produced. Bronfenbrenner has obtained similar results. Various authors in recent years, more particularly Zinsser, have discredited to a considerable degree the value of

the Abderhalden reaction. Nevertheless, as pointed out by Wells, "whatever may be said concerning the specificity of this reaction, there undoubtedly does commonly occur a greater amount of proteolysis in such mixtures with the specific antigen than if some other protein is present." Elsesser, working in Wells' laboratory, using Osborne's purified vegetable proteins, showed that "there is an obvious tendency for a substrate to react more often and yield stronger reactions when tested against its homologous immune serum, than when tested against a heterologous immune serum. We cannot afford to overlook the important fact that racemized proteins, which are characterized by being incapable of attack by enzymes *in vitro*, or of being digested and metabolized *in vivo*, are also incapable of serving as antigens, although derived from proteins which in the original state are highly antigenic." (Wells.[3])

The experiments of Friedmann and Isaak, and of Weichhardt and Schittenhelm make it appear "that, as measured by nitrogen output, the cleavage of foreign protein injected into specifically sensitized, or immunized, dogs occurred with much greater energy and speed than occurred in normal animals after first injection." (Wells.[3])

It has thus been established that proteolysis of specific antigens does occur when they are treated by immune serum. It is also known that intermediate products of protein cleavage are irritant.

Heilner,[4] basing his opinions upon the result of experiments, which proved that although the prompt metabolism of serum protein when fed by the mouth is evidenced by the appearance of its nitrogen in the urine, this is delayed for several days if introduced parenterally, assumes that the organism gradually responds to the introduction of such foreign proteins into the blood stream by the production of enzymes which are not ordinarily present therein, but which are adapted to disintegrate the new protein.

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[3]Wells: Physiological Reviews, 1921, i, 44.
[4]Heilner: Ztschr. J. Biol., 1912, lviii, 332.

That the process of parenteral digestion (or autolysis *in vitro*) results in the formation, by dissociation, of new compounds and not merely the liberation of a preformed toxic substance, is indicated by the similarity of the symptoms induced in animals by the introduction of split products prepared according to the method of Vaughan or by Rosenow's method of autolysis as well as the *in vivo* reaction of anaphylaxis following the injection of various proteins.

In Danysz's opinion, the immunologic reaction is the manifestation of parenteral digestion. Anaphylactic phenomena, he believes, occur as the result of flocculation or precipitation of antigen under the influence of antibody.[5] Active immunity, in his opinion, is a state of resistance of the organism to a certain part of the cell substance of infecting bacteria which is rapidly and easily digestible. It will be complicated by an anaphylactic hypersensitiveness whenever the compound of antigen with antibody is insoluble and, therefore, more or less difficult to digest. There will be no hypersensitiveness when this compound is soluble and neutral.

A true and lasting immunity would consist in rendering the organism refractory to anaphylactic hypersusceptibility; that is to say, to render the organism capable of completely digesting bacteria or products of bacteriolysis.

In the author's opinion, such true immunity is, in fact, exhibited when in consequence of repeated injections of such bacteria or other antigens, as are subject to lysis alone, the development of second order antibodies has been stimulated. Under such conditions the tissues are rendered tolerant to the irritant products of partial degradation of the bacterial protein and complete cleavage of the latter is accomplished.

Objections to the Theory of Parenteral Digestion

Although I am of the opinion that the objections hitherto raised to the theory of parenteral digestion do not suffice to

[5]It must be pointed out in this connection that the majority of immunologists exclude as examples of anaphylactic phenomena, all tissue reaction that are manifestly the result of flocculation and consequent vascular occlusion. The essential weakness of Danysz's contribution is, in my opinion, due to the fact that whereas all antigenic substances are colloids, Danysz assumes that all colloids act as antigens. In the great part of his experimental work he has employed non-protein "antigens."

disprove the hypothesis, I think it proper that the more important of the data which appear to indicate that this may, in fact, not be the proper explanation of the immunologic process should be included in this volume. It is of interest to bear in mind that, of the large number of investigators in recent years, few have made any suggestions as to what other mechanism may be responsible for the phenomenon of anaphylaxis. If we discard, says Wells, "the anaphylatoxin theory of anaphylaxis, we are left without any explanation of the very striking phenomena of anaphylactic shock, for no satisfactory substitute hypothesis has been proposed."

An important series of experiments, which cast a definite doubt upon the assumption that "anaphylatoxin" is produced by decomposition of the antigen,[6] proved that, by the treatment of normal serum by numerous insoluble substances, such as barium sulphate and kaolin, as well as bacterial cell bodies, a toxic substance is produced which appears to be identical with those developed by Friedberger's method. Experiments of this type have been carried out by Keysser and Wassermann, and by Bordet. The experiments of the latter author, in the treatment of fresh guinea pig serum by agar, are of the greatest importance. Similar experiments have also been performed by Jobling and Petersen, and by Novy and DeKruif.

Bordet has shown that the toxic substance, which is produced when normal guinea pig serum is mixed with agar, is characterized chemically by exactly the same alterations as occur when an immune serum is incubated with its antigen.

In 1917 Novy and DeKruif[7] reported a series of investigations, made by them on the nature of anaphylatoxin. They found that a disturbance similar to anaphylactic shock can readily be produced by the addition of almost any alien substance to a serum, whether in the living animal or in the test tube. The substances which may be successfully employed

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[6]The author particularly desires to point out that the adoption of the hypothesis of the presence of two substances, anaphylactic (first order) antibody and tolerant (second order) antibody, in the tissue of the immune animal or individual, is not dependent upon the solution of the problem as to whether the reaction between these antibodies and their protein antigen is in fact a cleavage of the latter or not.

[7]Novy and DeKruif: Jour. Am. Med. Assn., 1917, lxvii, 1524.

include bacteria, trypanosomes, organ cells and extracts, peptone, agar, starch, inulin, kaolin, silicic acid, barium sulphate, diverse salts and even distilled water. The result is a nonspecific anaphylactic shock.

These authors conclude that the poison produced does not come from the substance introduced, but that its matrix is a normal constituent of serum, and that the substances which convert it into anaphylatoxin act merely as inducers of accelerators of the reaction. Since shock blood is incoagulable, they believe that this inducing substance also reacts with fibrinogen.

They found that all bloods are toxic in the precoagulation stage. Thus, the blood of a normal rabbit, when rapidly transfused into the vein of a guinea pig, is usually harmless in a dose of 3 c.c. If, however, the blood is held in the syringe for three minutes before being injected, it becomes fatally toxic. The effects produced are those which characterize anaphylatoxin activity. The production of "anaphylatoxin" varies in different species of animals and in different individuals. The two reactions—blood coagulation and blood intoxication, in the opinion of Novy and DeKruif, are twin phenomena in which labile substances undergo intramolecular rearrangement. In the one case, the insoluble fibrin is produced, and in the other, the soluble "anaphylatoxin."

Jobling, Petersen and Eggstein[8] consider that the manifestations of acute anaphylaxis are brought about by the cleavage of serum proteins and proteoses to the peptone stage through the action of a nonspecific protease. They explain the mechanism of sensitization and anaphylactic shock in this way: "Serum ferments are practically unaltered by the primary injection of foreign protein. During the course of sensitization, the injection of antigen is followed by the mobilization of a nonspecific protease which increases in rapidity as the maximum period of intensity is reached. Acute shock is accompanied by the instantaneous mobilization of a large amount of nonspecific protease, decrease in antiferment, increased

[8]Jobling, Petersen and Eggstein: Jour. Exper. Med., 1915, xxii, 401.

in the noncoagulable nitrogen of the serum, increase in the amino acids and a primary decrease in serum proteoses. Later, there is a progressive increase in the noncoagulable nitrogen, in proteoses, and in serum lipase. The specific elements lie in the rapid mobilization of a nonspecific protease and the colloidal serum changes which bring about the change in the antiferment titer.''

In other words, substances such as those mentioned above remove or absorb, the antienzymes which are normally present in the blood. In consequence, of this alteration in the relationship of antiferments to normal ferments in the serum, the latter bodies in the fresh serum act upon the serum protein with consequent cleavage of the protein molecule and the production of irritant split products.

The fact that toxic split products are produced by autolysis of the tissue proteins when treated by various inert substances, which appear to remove antiferments from the body fluids, does not of necessity disprove the hypothesis that anaphylaxis is due to the cleavage of the protein antigen by the action of sensitizing substances plus alexin. There can be no doubt that wherever proteolysis takes place toxic substances may be formed. This is proved by the work of Vaughan and by Friedberger's and Friedemann's experiments and by the results of protein cleavage by means of trypsin.

The late Richard Weil[9] opposed the views embodied in the theory of parenteral digestion most urgently. According to his interpretation of such experiments, it is not the antigen but the antiserum which undergoes chemical alteration when antigen and antibody react *in vitro*. The antiserum, itself, undergoes autolysis. He ignores all those experiments which have shown that it is possible to induce anaphylactic shock by the simultaneous introduction of antigen and antiserum.

Jobling has shown that the blood of dogs during anaphylactic shock exhibits definite chemical evidence of protein disintegration. This fact Weil disposes of by stating that the chemical changes are simply the harmless bi-products of the

[9]Weil: Jour. of Immunol., 1916-17, xi, 525.

anaphylactic reaction on the sensitized liver, and are comparable to those which occur in chloroform or phosphorous poisoning.

By an ingenious set of experiments Weil[9] injected the blood of dogs in the height of anaphylactic shock into normal dogs. No symptoms of any kind resembling anaphylaxis occurred. These experiments appear to prove that the blood of the dog in the anaphylactic state does not contain toxic substances. If such a toxic property of the blood during anaphylaxis be proved to exist, proof of the production of an irritant in consequence of the reaction between antibody and antigen will have been supplied. Negative results, however, cannot in the author's opinion, be interpreted as proof that a toxic substance has not been produced in the tissues.

Zinsser is of the opinion that "if an antigen participates at all in furnishing the substratum for the (anaphylactic) poison, this is probably less important than that furnished by the animal's own proteins.[10] However, this does not weaken the importance of the knowledge that antigen-antibody reactions in the presence of normal serum, and certain antigens in the presence of normal serum alone, induce a reaction in the course of which such irritants are formed. And the fact that they can be produced experimentally in the peritoneal cavity of a living guinea pig, renders their participation in such reactions in the animal body a likely assumption."

Dale believes that the chief objection brought to bear by his experiments upon the production of a toxic cleavage product, as the substance responsible for the anaphylactic reaction, is the time relation. The specific antigen acts on the isolated plain muscle with as little delay as a direct stimulant. If the effect produced were due to the action of a ferment, he says one would expect a gradual onset, and the gradual response to a maximum. But the onset is sudden and the rate of

[10]Although it is possible to imagine that in constitutional reactions such as anaphylactic shock the substrate from which the tissue irritant is produced may be derived from the animal's own body proteins, this cannot be the cause of phagocytosis of the particulate protein in the hypersensitive animal.

progress to the maximum is apparently limited only by the contraction rate of the plain muscle.

Again Dale [11] points out that the possibility of the production of a poison by parenteral digestion is not by any means excluded by the demonstration of the specific response in tissues freed from blood, since, given the demonstrated attachment of antibody to muscle-cells, or its incorporation into them, a poison produced by the interaction between antigen and the antibody so situated, would arise under conditions ideal for the exhibition of its activity.[12]

Explanation of the Phenomenon of Tolerance

"Immunity" (tolerance) is a state of relative insusceptibility to anaphylactic shock. "It is characterized by an increase in the amount of free anaphylactic antibody. Whether the insusceptibility of an immune guinea pig is due to an excess of free anaphylactic antibody, to the formation of another free antibody, or to specific changes in the somatic cells, is an open question." (Anderson and Frost.)

The animal which is not susceptible to anaphylactic shock when treated with moderate doses of antigen, but whose serum confers passive sensitization upon normal animals, is said to be tolerant. This is the state which had been referred to by different observers as immunity, or antianaphylaxis. The author believes, however, that the employment of the term "tolerant" is more suggestive and accurate.

It has been proved experimentally that not only is a frequently treated animal tolerant, but that such tolerance may be transmitted passively to normal animals, or even to hypersensitive animals, by injection of sufficient quantities of serum from a tolerant animal. It is possible to passively sensitize normal animals by the transference of blood serum or tissue fluid of a treated animal. In order to passively sensitize an animal with the serum of another animal, which had received but one injection of antigenic protein, and which is, therefore,

[11] Dale: Jour. Phar. and Exper. Therap., 1912-13, iv, 167.

[12] This point of view has been amplified by the author on page 144.

extremely sensitive to small doses of the same antigenic protein, it is necessary to employ a large proportion—one-half to seven-eighths—of the total blood serum. On the other hand, the serum of animals which have received repeated large doses of antigenic serum, and which are consequently tolerant, suffices, in minute amounts—0.01 to 0.6 c.c.—to confer passive hypersensitiveness upon normal animals.

The paradoxical phenomenon is thus noted that the animal in which hypersensitiveness is more readily demonstrated contains in its serum a much smaller amount of anaphylactic antibody than does the animal in whom proof of hypersensitiveness is obtained only with difficulty, i.e., by the employment of relatively large toxic or "exciting" injections.

If an explanation of the phenomenon of anaphylaxis is difficult, that of tolerance is equally so. Friedberger early suggested that remnants of the antigen (injected protein) persist in the tissues and that these render the animal practically refractory. This view has since been very generally discredited by practically all observers including Friedberger himself. As is but natural, it has been suggested, by those who look upon anaphylaxis as essentially the result of parenteral digestion of proteins, that when large amounts of ferment are present in the blood and tissues, hydrolysis of the foreign protein, when the latter is reinjected, advances rapidly to a point which results in the production of nontoxic end products. Friedberger has shown that the poison, prepared by him, may be destroyed by too long digestion or by an excess of anaphylactic serum. According to the conception advocated by Friedberger, this diminution in toxicity is due to the more complete activity of the antibody which is responsible for precipitin formation and for the development of the anaphylatoxin. The author believes that the assumption of the elaboration by the tissue cells of a second order of antibody is supported by the facts at our disposal more adequately than is the hypothesis which conceives the first order (anaphylactic) antibody to be potent to accomplish complete dissociation of the protein molecule.

The author believes that in the light of our present information a larger number of disease phenomena can be explained if it be assumed that the process is essentially one in which a substance is produced which reacts with the product of the anaphylactic (first order) antibody-antigen reaction in such a way that it is rendered innocuous to the tissues.

If it be assumed that the anaphylactic state is due to the presence in the animal body of specific proteolytic antibodies, which so react with their specific substrates that irritant products are formed, it seems reasonable to suppose that the insusceptibility to anaphylactic shock demonstrated by tolerant (immune) animals may be due to a further exhibition of the property of parenteral proteolysis.[13]

The hypothesis suggested by the author regarding the relationship of the anaphylactic to the tolerant state is as follows: The parenteral introduction of protein antigens into the animal body is followed by the elaboration of specific antibodies which so react with the complex protein molecule that an irritant product is formed. This poisonous product is, perhaps, identical with the poisonous split product of Vaughan, or the anaphylatoxin of Friedberger. In consequence of the presence in the tissues of this first order antibody, the animal is hypersensitive to the re-injection of the specific protein, as the result of whose primary injection the production of antibodies was induced. Subsequent introduction of antigen, in sublethal doses, results in the stimulation of a second order of antibody, the activity of which renders the irritant, produced by the first order antibody-antigen reaction, harmless to the tissues.

According to the author's hypothesis, the animal becomes hypersensitive by virtue of the presence in its body fluids and its tissue cells of the first order antibody, which so reacts with its specific antigen that a substance, which acts as an irritant to certain tissue cells, is produced. The tolerant animal is

[13]This may be due to the activity of an enzyme-like peptolytic substance. Such peptone-splitting ferments have not, heretofore, been demonstrated in the serum, although Smith has identified a ferment capable of dissociating the polypeptid,—glycyltryptophan. (Jour. Am. Med. Ass., xix, 539.)

insusceptible to anaphylactic shock in consequence of the presence in its tissues of a second order antibody, which is potent to so act upon the product of the reaction between the first order antibody and its antigen, that it no longer acts as an irritant to tissue cells.

Two alternatives in explanation of the tolerant state seem possible; either the cells, which, in the anaphylactic or normal animal, are susceptible to the injurious action of the "anaphylatoxin,"—lose their receptors and become incapable of absorbing, and hence are not injured by the irritant; or, there is developed an antibody capable of either neutralizing, by a process of synthesis, the anaphylatoxin, or of further dissociating it into harmless substances. Loss or exhaustion of anaphylactic antibodies, undoubtedly, does occur. This is the condition which has been discussed under desensitization.

Vaughan refers to the condition as one of tolerance, but attempts no explanation of its possible nature. He states, specifically, in speaking of his toxic split product, that when repeatedly injected in nonlethal doses the antibody does not elaborate an antibody—an antitoxin. He says, however, that the phenomenon requires further study, employing the method of passive immunization. I believe that experiments[14] which were published in 1914 supply certain data believed by Vaughan to be necessary for a more adequate understanding of the subject.

The difficulty which is experienced in provoking anaphylactic shock in passively sensitized animals without the lapse of a certain interval of time—six to twenty-four hours—has been interpreted, by many observers, as indicating that a reaction between antigen and circulating antibodies cannot induce symptoms of anaphylactic intoxication. The experiments reported by myself and others (Chapter IX) show that it is possible to induce anaphylactic shock by the action of the specific antisubstance and the antigen while both are circulating in the blood stream.

The author's[15] experiments were undertaken in the hope

[14]Gurd: Jour. Med. Research., 1914, xxvi, 205.
[15]Gurd: Jour. Med. Research., 1914, xxvi, 205.

of proving by direct methods the presence, in the serum of so-called immune animals, of a substance capable of protecting the sensitized animal against manifestations of anaphylaxis. The results of these experiments proved the possibility of passively transferring the tolerant state to normal animals. Inasmuch as these experiments which were published in 1914 are not generally known, several protocols are republished.

EXPERIMENT A.137.—A series of guinea pigs, weighing about 340 grams each, were injected with immune rabbit serum (38) of such a titer that 0.6 c.c. injected intraperitoneally sensitized animals (250 grams) so that upon the following day 5 minims of sheep serum caused typical anaphylactic death; 0.5 c.c. of the rabbit serum was insufficient to passively sensitize so that death supervened, although marked symptoms developed.

GUINEA PIG NUMBER	FIRST INJECTION	INTERVAL	SECOND INJECTION	RESULT
109	.6 c.c., I.R.S., i.p.	24 hours	.35 c.c., i.v., S.S.	Marked symptoms Recovery.
110	.6 c.c., I.R.S., i.p.	24 "	.45 c.c., i.v., S.S.,	Typical anaphylactic death 6 minutes.
103	4.0 c.c., I.R.S., i.p. 12 hours later 4.5 c.c., i.p., I.R.S.	25 "	Sheep Serum .5 c.c., i.v.	Slight malaise.
99	1.0 c.c., I.R.S., i.p.	7 days	.3 c.c., S.S., i.v.	Very severe symptoms.
100	1.0 c.c., I.R.S., i.p.	7 "	3.5 c.c., I.R.S., i.v., .4 c.c., S.S., i.v.	Very slight symptoms.
147	.5 c.c., I.R.S., i.p.	24 hours	.5 c.c., S.S., i.v.	Typical death 4 minutes.
148	.75 c.c., I.R.S., i.p.	24 "	.5 c.c., S.S., i.v.	Typical death 3 minutes.
149	2.5 c.c., I.R.S., i.p.	24 "	.5 c.c., S.S., i.v.	Marked symptoms Recovery.

I.R.S.—Immune Rabbit Serum;
i.p. —Intraperitoneal injection;
i.v. —Intravenous injection;
S.S. —Sheep Serum.

In the foregoing experiments the animals were allowed to pass through the so-called "incubation stage." As is seen, the injection of larger quantities of serum was sufficient to protect the animal (103) against the anaphylactic shock which followed in the other two animals.

The following protocol shows the effect of immediate toxic injections in passively sensitized guinea pigs; it is even more striking, although none of the animals died:

111. 280 grams. Received .9 c.c. of rabbit serum (38) i.v., within two minutes 7 minims of sheep serum i.v.—marked immediate symptoms for ten minutes—rapid recovery.

113. 275 " Received .5 c.c. of rabbit serum (38) i.v., within two minutes 7 minims of sheep serum i.v.—immediate onset of marked symptoms progressing to convulsions and paralysis, apparently dying, but recovered.

112. 280 " Received 2.75 c.c. of rabbit serum (38) i.v. within two minutes 7 minims sheep serum i.v.—no symptoms.

The following series of experiments is quoted from Weil. It also shows the protective effect of larger doses of immune rabbit serum injected into normal guinea pigs:

GUINEA PIG	SENSITIZING INJECTION INTRAVENOUSLY	TOXIC INJECTION NEXT DAY INTRAPERITONEALLY	RESULTS
	c.c.	c.c.	
1	0.3	2.5	No symptoms.
2	0.5	2.5	After 20 minutes paretic, atactic, mild convulsions. Recovered in two hours.
3	0.5	2.5	Similar to above.
4	0.7	2.5	Respiratory and cutaneous symptoms. Severe prostration. Died during day.
5	0.8	2.5	Died in 40 minutes.
6	1.0	2.5	Immediate dyspnea and paresis. Convulsions; death in 12 minutes.
7, 8	2.0	2.5	Very mild symptoms.

These results were interpreted by Weil as indicating that "larger injections of the serum leave so considerable a residue of antibody in the circulating blood that the animal is protected against the anaphylactic effects of peritoneal injections of antigen." According to the hypothesis brought forward by the author, this protective effect of larger doses of transferred serum is due to the presence of tolerant, or second order, antibodies in sufficient quantity to protect the animal against the effect of the interaction of antigen and first order antibody.

These experiments prove that the blood serum of immune animals contains, not only sensitizing bodies, but also some substances which are potent to protect the animal from the effects of the reaction between anaphylactic (first order) antibody and antigen. Theoretically, this substance might consist of an antiferment, an excess of the "anaphylactic ferment" which latter must, in this case, be assumed to be capable of more or less completely digesting the toxic split product into simpler harmless substances, or there is produced, following the reinjection of sensitive animals, a second type of antibody which exhibits the property of inactivating the irritant product of the reaction between first order body and antigen.

The likelihood of antiferment action being responsible for the protection induced is very remote; there is little to support this view and many observations would indicate that it is not the correct one. That an excess of the same enzyme-like substance, which may be responsible for the further cleavage and consequent detoxication of the poison, seems more reasonable, but this view also fails to explain certain phenomena which will be referred to presently.

The explanation of this phenomena, offered by exponents of the "cellular" theory, is as follows: They assume that the sensitive animal is such by virtue of the production of sessile receptors and that the sensitizing bodies present in the serum are of the nature of protective antibodies, in the sense that these free circulating bodies bind the introduced antigenic protein and thus make it impossible for the antigen to

affect the fixed receptors attached to the cells. The chief objections to this hypothesis are:

(1) That such a theory assumes an essential toxicity for complex proteins—albumins and globulins.

(2) That the mixture *in vitro*, or *in vivo*, of serum, containing free receptors and antigenic protein, results in the formation of a toxic substance.

(3) That the number of units of sensitizing bodies—free receptors—in the blood serum is much greater than is the capacity of the animal for withstanding injections of antigenic protein.[16]

The humoral theory requires the assumption of an intermediate toxic product resulting from the action of antibody upon antigen which, secondarily, affects the cells.

[16]Weil has shown that about 30 or more units must be present in order to protect the fixed cellular receptors.

CHAPTER XVI

ALLERGIC REACTION

When the hypersensitive animal receives an injection of a suitable dose of the specific antigenic protein, to which it has been sensitized, the nature of the reaction which is exhibited is dependent upon the route of administration. If injection of a soluble protein be made into a vein, severe symptoms, respiratory in the case of the guinea pig, or circulatory in the case of the dog, immediately take place and the animal dies. If the protein be injected into tissues such as the muscles, serous cavities, or subcutaneous tissues, in addition to constitutional manifestations of intoxication, focal evidences of irritation may be exhibited. It is possible, in this way, by means of the subcutaneous injection of either soluble or particulate proteins, to induce all the manifestations of inflammation, i.e., hyperemia, swelling, and leucocyte accumulation. In the human tissues, well marked examples of acute cellulitis are readily provoked, in individuals hypersensitive to horse serum, by the subcutaneous injection of small doses of this antigen.

In the previous pages we have studied chiefly the systemic reaction which occurs in sensitized animals following the parenteral injection of soluble proteins. Coincidentally with the study of this reaction by Rosenow and Anderson, by Richet, and by Otto, von Pirquet and Shick had observed and studied a phenomenon to which they applied the name "allergy" (*allos*—altered, *ergeia*—reaction). The essential characteristics of the allergic reaction are that, when antigenic protein, whether particulate or insolution and whether viable or inanimate, is injected into the soft tissues of hypersensitive individuals or animals, there is an alteration in the morphologic reaction which occurs, as compared with that exhibited, following similar parenteral introduction into nor-

mal animals. The fundamental alteration consists of a shortening or elimination of the incubation period, and if viable antigen be employed the degree of reaction is minimized.

Von Pirquet's original statements were based upon a study of vaccinia vaccination and serum sickness. He noted that the individual who has not previously been vaccinated or been the subject of smallpox infection, reacts to the inoculation of cowpox virus in a typical manner. One important characteristic of this typical reaction is that a definite number of days elapse before the reaction becomes evident. In such individuals, not only is the reaction delayed but, once it has commenced, it continues for a number of days so that a definite cycle of tissue changes which last for a period of two weeks or longer is noted.

On the other hand, if an individual who has been previously successfully inoculated, is subjected to a second vaccination, the onset of the stage of hyperemia is developed very soon after the inoculation of the virus. The alteration in the time of onset of the reaction may be such that but one or two hours intervenes between the introduction of the virus and the onset of the inflammatory reaction. Furthermore, the characteristic series of changes, namely, vesiculation, pustulation, and scab formation, do not take place. Von Pirquet recognized in this phenomenon a principle that has become one of the utmost importance both in the diagnosis of infective conditions and in our appreciation of disease phenomena.

Briefly stated the essential characteristic of the phenomenon studied by von Pirquet is that, as a result of a previous injection of antigen, or an antecedent infection, some change is brought about in the body tissues as a result of which, if the individual be reinoculated with living microorganisms, reaction takes place much more promptly than in the normal individual. The severity of the reaction is, moreover, less marked. The incubation period is shortened and the reaction is minimized.

In the normal individual the introduction of a pathogenic microorganism is immediately followed by its proliferation.

Multiplication of the injected units continues, until such time as the body develops a sufficient number of antisubstances to stimulate a reaction. Von Pirquet believes that as a result of the reaction between virus or antigen and antibody, there is produced a toxic substance which stimulates vascular and cellular reaction. Since, in a previously inoculated individual, there already exists at the time of the second introduction a considerable quantity of antibody, this combination of antigen and antibody with the formation of a toxic product, supervenes very soon after introduction of the virus. In consequence a morphologic inflammatory reaction is stimulated almost immediately. Since this reaction takes place very soon after inoculation, proliferation of the virus *in situ* has not had time to take place. A prolonged or marked reaction is, therefore, not necessary in order that the infectious agent may be eradicated.

Since von Pirquet's original contribution upon this subject, numerous observers have proved that clinical manifestations of the allergic phenomenon are very common. It is the basis of the cutaneous reaction to tuberculosis, to which the name of von Pirquet is applied, the luetin reaction of Noguchi, the gonococcus reaction of Irons, the mallein reaction in cattle, and tests for protein hypersensitiveness in such conditions as asthma and pollenosis. I employ the intradermic allergic reaction as a routine in determining the dose, and time interval, in the employment of vaccines for therapeutic purposes (See Chapter XXV).

If two individuals, one of whom was vaccinated against cowpox two years previously, and the other has never been infected with either vaccinia or smallpox, be vaccinated at the same time, the phenomena which develop in the two cases differ in a typical manner. At the end of twenty-four hours the previously inoculated person exhibits on the arm a small elevated inflamed and itching scratch, whereas the newly vaccinated person shows but a trivial scab unaccompanied by evidences of inflammation. It appears at this time as though the previous vaccination had rendered the individual more

susceptible to vaccinia virus. The subsequent course of events, however, shows that this is not the case. The papule on the previously vaccinated case rapidly subsides and disappears, whereas the normal individual exhibits, after the lapse of several days, a larger inflamed area which progresses to vesicle formation and pus accumulation. In the first individual no fever or other constitutional manifestation of disease is exhibited, whereas the previously unvaccinated patient manifests moderately severe symptoms of intoxication.

Von Pirquet's Studies upon Vaccinia[17]

Since the inoculation of viable pathogenic microorganisms is rarely justifiable in the human subject the experiments of von Pirquet and Shick are of the greatest importance. The alterations in the reaction which occurs when hypersensitive individuals are subjected to vaccinia virus inoculation have been thoroughly studied by these observers.

Of all infectious diseases in man, cowpox is best suited to exact clinical and experimental study. The first vaccination, in healthy children, shows a constant symptom-complex. Some minutes after the vaccination, a traumatic reaction, in the form of a very slight redness, appears. This reaction persists for approximately twenty-four hours and leaves a small scab surrounded by normal skin. On the third or fourth day, a small red papule appears, which indicates the beginning of the specific reaction. Between the fourth and the sixth days, the middle portion of the papule becomes more elevated, the outer part becomes flat, and forms a narrow red circle around the papule. From now on, the papule increases in size quite regularly, about 1 millimeter a day, and the solid papule is transformed into a blister. The aureola remains of the same width and is protruded only by the extension of the papule.

Between the eighth and the eleventh days, the aureola increases to a large slightly elevated inflammatory plaque. The papule ceases to grow and becomes yellow. Between the

[17]This section is abstracted with but few alterations from von Pirquet's article. Arch. Int. Med., 1911, vii, 260.

eleventh and the fifteenth days the aureola reaches its highest development and then disappears slowly, whereas the papule dries and a large scab falls off, leaving a scar. During the time of aureola formation general symptoms appear in association with this local reddening. The special features are fever and leucopenia.

On revaccination, characteristic changes of reactivity are seen. If daily vaccination be made for a fortnight upon the same individual, the allergy evinces itself most distinctly. The most striking feature is that this inflammation appears on all the vaccination points simultaneously. Although the inoculations were made on successive days, the aureola develops around all the vaccination points at the same time, that is, at the time when its development is due on the first vaccination point. From now on, the papules also of the later vaccinations stop growing, as does the papule of the first vaccination. In those vaccinations which have been made from this time on, the state of papule formation is no longer reached. It is thus seen that if the tissues have been recently exposed to the presence of antigen, the reaction which accompanies reinoculation is exhibited at once. This is the "immediate reaction" of von Pirquet's terminology.

Another type of reaction occurs, when a few months intervene between the primary and second inoculation. This is called by von Pirquet the "early reaction." In this reaction a papule is formed, which reaches its maximal development in twenty-four hours.

If several months or years have elapsed between the first and second vaccinations this type of very early reaction is replaced by another. Here the reaction occurs somewhat later, within the second day, reaching its maximum on the third or fourth day ("torpid early reaction").

The longer the interval of time between the first and second vaccinations, the more frequently are intense reactions noted, perhaps, even with the formation of papule and aureola. Nevertheless, these reactions following reinoculation still show some difference from a primary vaccination, inasmuch as the

aureola develops more promptly and the growth of the papule is interrupted at an earlier stage ("accelerated reaction").

Comparing the sum total of the events of a first vaccination with those of revaccination, the individual vaccinated for the first time suffers from extensive local inflammation, fever, and other general symptoms. The revaccinated person overcomes the infection with a very slight local reaction a few millimeters in size. But observing the reaction on the day following the vaccination, it is evident that the revaccinated is hypersensitive because at this time the person vaccinated for the first time does not show any reaction, while the revaccinated individual responds with a local inflammatory process. "Repeating the vaccination very frequently on the skin of my [von Pirquet's] lower arm, I finally became hypersensitive to such a degree that within twelve hours a papule of 9 millimeters in diameter developed, a size which after a first vaccination, is not reached before the seventh day."

Von Pirquet noted that the size of the allergic reaction depends quantitatively upon the amount of vaccinia used. With fresh diluted lymph early reactions, 3 centimeters in diameter, were obtained with the formation of vesicles. When the lymph is diluted, the size of the reactions is less. The phenomena which accompany revaccination of the previously vaccinated person, differ in this respect from those of the first vaccination.

When the individual is vaccinated for the first time the amount of vaccine does not influence appreciably the size of the reaction. The previously inoculated individual is sensitized to the vaccinia protein. In consequence the tissues immediately react to the introduction of the antigen. The larger the amount of antigen introduced the more extensive is the inflammatory reaction which is stimulated. The normal individual, on the other hand, who is not hypersensitive to the virus protein, does not react immediately. The virus continues to proliferate so that, by the time the incubation period is past, and the tissues become hypersensitive, a sufficient amount

of virus protein antigen had been produced to necessitate an extensive area of reaction.

Von Pirquet next directed his attention to the study of the allergic reaction in other diseases, more particularly tuberculosis, and has been successful in establishing the alteration of the reaction on the part of the tissues to the reinjection of antigen as a biologic law. He showed that not only does the body react in an altered manner to inoculation by a viable microorganism, but that the previously infected and hence sensitized individual will react, morphologically—as shown by papule formation and other evidences of inflammation, within a very short period—one to thirty-six hours—after the introduction into the skin of nonviable bacterial proteins. The normal individual, whose tissues are not hypersensitive to the bacterio-protein, does not react in any visible fashion to the introduction of moderate quantities of the antigen (tuberculin).

Weil[18] describes an interesting local allergic reaction, which occurs in the liver of hypersensitive dogs. A dog was sensitized by the intravenous injection of 5 c.c. horse serum. After an interval of three weeks, the animal was put under ether and the abdomen opened by a median epigastric incision. The surface of the liver was punctured by a very fine needle attached to a hypodermic syringe, and a drop of a 10 per cent solution of horse serum was injected, just below the surface. Almost immediately the point of injection became the site of an intense and sharply localized congestion, measuring perhaps ½ inch in diameter, and over this area the surface of the liver was distinctly raised. The duration of this reaction was not accurately determined by Weil; it did persist for more than one-half hour.

Another dog was sensitized by the intravenous injection of 5 c.c. of horse serum. Two weeks later the dog was etherized and the liver exposed by a median epigastric incision. A subsurface injection of a minute amount of horse serum into the

[18]Weil: Jour. Immunol., 1916-17, xi, 538-540.

liver provoked an immediate congestive reaction. Into the right branch of the portal vein 0.5 c.c. of horse serum was then injected. The right lobe of the liver at once became intensely congested, while the left lobe showed no change. Blood was aspirated from the jugular vein five minutes later and was found to be incoagulable. After fifteen minutes, although etherization had been discontinued at the moment of portal injection, the animal appeared to be in shock, and the carotid pulsation was very small. The dog was killed and the organs examined. The large abdominal veins were distended, but the abdominal viscera were not congested, with the exception of the liver. The right half of the liver was deeply congested, cyanotic in color, and firm to pressure, and the cut surface bled freely. The left half was only slightly, if at all, congested.

A similar and very interesting type of local allergic reaction has been noted by Auer.[19] The essential difference between the reaction which follows injection of antigen into soft tissues and Auer's reaction is that in the latter the antigen was brought in contact with the local tissues by what may be called a process of autoinoculation.

Auer noted, in testing the sensitiveness of dogs that had been treated with horse serum some years previously, that a peculiar edema developed at the site of the operation wound in the inguinal region. There occurred about two days after the test a fairly extensive thick brawny edematous mass of tissue; no discharge from the wound occurred. Auer assumed tentatively that the following reaction had occurred. The foreign protein (horse serum) was circulating, due to the reinjection. A certain amount of this protein passed into the tissues adjoining the wound during the development of the ordinary wound edema which always follows an operation. As the dogs were sensitized to the foreign protein an anaphylactic (allergic) reaction to the local autoinoculation to the horse serum occurred. Auer confirmed these findings by a very interesting observation in which he induced a moderate

[19] Auer: Jour. Exper. Med., 1920, xxxii, 427.

inflammatory reaction in the rabbit's ear by means of xylol. Ordinarily this reaction disappears in a short time without serious injury to the skin. The effects are quite the same in a rabbit that has been sensitized to a foreign protein, but in rabbits that have been sensitized and then reinjected with the same protein the xylol commonly produces a violent reaction, with exfoliative dermatitis followed by dry gangrene of the tips of the ears. The explanation of this striking effect seems to be simple. The slight inflammation produced by the xylol leads to a certain amount of inflammatory exudate. In the sensitized animals which have been recently reinjected with the sensitizing protein, the blood contains free antigen; a minute amount of this antigen is poured out into the tissues with the exudate. Here it produces a local reaction in the sensitized tissues, quite the same as if it had been locally injected. Presumably similar effects could occur in any other tissue or organ. "The importance of this observation lies in the recognition of a hitherto unappreciated mechanism by which anaphylactic reaction may be caused." (Wells.[20])

Factors Determining Specific Types of Allergic Reaction

When normal rabbits are injected with killed cultures of the staphylococcus aureus of the bacillus tuberculosis, practically no reaction of any sort is noted. It is of comparatively little importance whether the bacterial suspensions are injected into the blood stream, intraperitoneally, or into the subcutaneous tissue. Experiments of this nature prove the absence of any very potent essential toxin on the part of either of these microorganisms. If, on the other hand, two rabbits are inoculated with living cultures injury to the animals is noted and there are exhibited manifestations of reaction on the part of the tissues. After an interval, which is known as the incubation period, the rabbit which received an inoculation of the staphylococcus aureus shows focal and systemic reactions of an acute type. The reaction is evidenced

[20]Wells: Physiol. Review, 1921, i, 81.

by increase in pulse rate, pyrexia, leucocytosis; focal destruction of tissues, and pus cell accumulation at the site of injection occurs.

The other rabbit, which was injected with viable tubercle bacilli, remains apparently well for a much longer period than does the animal which has been injected with the coccus. After this longer incubation period a reaction occurs which is characterized not by polymorphonuclear infiltration of the part, but by necrosis and by the accumulation and proliferation of cells of the lymphoid and plasma, and "epitheloid" type. In other words a tubercle is formed in the tissues. The constitutional disturbances which accompany the focal reaction are less fulminant and less severe than those exhibited by the coccus injected rabbit, but persist for a longer period. They eventually lead, after a period of from five to eight weeks, to death of the animal in an extreme state of emaciation.

By another set of experiments it may be proved that although the normal rabbit does not react to injections of dead bacteria of either of the types employed in the above experiments, it is possible to induce similar reactions focally upon the part of the tissues, if, after an interval of two weeks following the introduction of a devitalized bacterial suspension into the subcutaneous tissues, a second injection of the same dead bacteria is made. The reaction which occurs is similar, in each case, to that which took place when the living microorganisms were employed for injection. There is, however, an absence of incubation period, and the reaction persists for a relatively short time.

These three sets of experiments prove that, although the staphylococcus aureus and the tubercle bacillus are not in themselves toxic to normal animals, there is developed by their presence in sensitive animals, a toxic substance. It is, furthermore, proved that the type of reaction which is stimulated on the part of the tissues, is specific for each of the two bacteria.

There is every reason for believing that the irritant substance which is responsible for the reaction is developed from

the bacterial protein.[21] That it is only developed in the presence of a specific substance in the body fluids or tissue cells is evident from the fact that if a rabbit which has previously received a dose of staphylococcus aureus be subsequently injected with devitalized tubercle bacilli, no reaction takes place. Similarly, if the bacillus tuberculosis suspension be first injected, no reaction takes place upon the subsequent introduction of the coccus. It is apparent, therefore, that because of differences in the morphology or chemistry of bacterial cells, toxic products of different irritative potency, or concentration, are liberated.

Other experiments indicate that no matter what the chemical composition of the protein molecules constituting the cytoplasm, the irritating substance produced as the result of interaction of the antigen and antibody is the same. It is, therefore, probable that, with the exception of specific toxins developed by certain bacteria during their growth (e.g., B. diphtheria, B. tetani), the irritative properties exhibited by bacteria in the tissues of sensitive animals is the result of an antigen-antibody reaction. It is also probable that no matter what the source of the protein antigen may be, the irritating substances so developed are identical in nature. We are led to the conclusion, therefore, that it is the physical (e.g., relative solubility, thickness of ectoplasm or the presence of capsule either mucoid or waxy), rather than the chemical, characteristics of individual species of bacterium which determine its relative irritative properties, and that consequently induce specific vascular and cellular reactions on the part of the tissues. In discussing the morphologic characteristics of bacteria in an earlier chapter, the importance of the waxy covering of the tubercle bacilli has been indicated.

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[21]Although I have considered the possibility of the irritant product being derived from the animal's own tissue proteins, I am of the opinion that the data available justifies the adoption of the view expressed in this paragraph as correct.

CHAPTER XVII

THE FUNCTION OF THE LEUCOCYTES IN IMMUNOLOGIC PROCESSES

In addition to the property of specific antibody production, the body has at its disposal a second process whereby infecting bacteria may be destroyed. This consists in the exhibition of phagocytic activity by various tissue cells.

The function of the polymorphonuclear leucocyte, or pus cell—so called since it is the most constant cellular component or purulent material—is two-fold, namely, phagocytic and secretory. If small particles of irritant protein substances, as, for instance, bacteria, be sufficiently irritating to stimulate the pus cell to activity, the latter throws out pseudopodia and engulfs the irritant body. Subsequently, it attempts to destroy and digest the ingested material in its cytoplasm by means of a ferment, present in its cell substances, known as leucoprotease.

Phagocytosis of inert material is accomplished by the mononuclear cells. This phenomenon is commonly noted in the lungs and bronchial lymph nodes in which are cells which take up carbon pigment.

If pus from a case of gonorrheal urethritis be examined microscopically, it is found to consist chiefly of polymorphonuclear leucocytes similar to those found in the blood. Many, or most of these cells are seen to contain in their protoplasm, larger or smaller, numbers of gonococci. Either these bacteria are within the cells as a result of their own growth and migratory activities, or the cells have engulfed the bacteria. That the latter alternative actually takes place is demonstrated by the fact that if a suspension of dead bacteria (e.g., staphylococci or gonococci) be injected into the subcutaneous tissues

in suitable subjects,[1] a small pus collection forms, the component cells of which are found to have ingested a certain number of bacteria.

The principles underlying the phagocytic reaction are perhaps not clear; there are, however, certain facts which appear incontrovertible. Of the possible factors which might influence phagocytosis there may be active one or more of the following:

1. The pus cells may have acquired an increased avidity for the type of bacterium used in the experiment as the result of some physiologic change in themselves.

2. Stimulated by some substance present in the serum the cells may be induced to function to an unusual degree.

The fact that cells from normal individuals and cells from immune persons, if washed with salt solution and treated with the same serum, exhibit equal phagocytic power disproves the likelihood of the former alternative.

3. It is possible that the bacteria are so affected, by some antibody in the serum, that they are rendered more likely to be attacked by the leucocytes. That this view is correct is proved by the fact that if the bacteria be washed, after having been exposed to the action of the immune serum, and be subsequently added to a mixture of leucocytes and fresh normal serum phagocytosis proceeds as in control experiments in which specific immune serum is employed.

It may, therefore, be assumed that phagocytosis of bacteria, when the latter are treated with immune serum, is due to an alteration in the bacterial cell bodies.

The change which takes place in the bacteria which are made the subject of experimentation may conceivably be of one or other of two forms. Resistance of the bacterial cell to ingestion may depend upon production by the bacteria of toxins, or substances of a similar nature, which prevent phagocytosis or

[1]The suitability of the subject depends upon his state of hypersensitiveness to the bacterial protein. In another chapter it is shown that there is reason for believing that it is only in so far as the individual's tissues are hypersensitive to the bacterial protein that the bacterial body becomes irritating to the tissues, and consequently stimulates reaction on the part of the body cells.

repel the leucocyte (aggressins).[2] On the other hand it may be that the normal bacterial cell body is not sufficiently irritating to stimulate the leucocytes to activity. In the terminology of Metchnikoff absence of phagocytic activity may be due to a "negative chemotaxis" exhibited by the bacterial, or merely lack of "positive chemotaxis."

The author believes that sufficient proof is available to justify the conception that phagocytosis of bacterial cells occurs in direct proportion to the irritant properties exhibited by the bacterial cytoplasm. As a rule variations in actual irritative property depends upon hypersensitiveness of the tissues to the bacterioprotein.

In consequence of the reaction between the first order antibody (anaphylactin) and the bacterial protein the relatively innocuous bacterial cell body is transformed into a highly irritant substance which stimulates leucocytic activity and leads to ingestion of the bacterial cell. Within the cytoplasm of the phagocytic cell more complete degradation of the protein and consequent dissolution of the bacterium takes place.

Certain experimental data, in which nonviable and nontoxic substances are employed, throw light upon the subject. If a slowly soluble nontoxic substance, such as catgut or animal charcoal, be broken up and injected into the subcutaneous tissues, or peritoneum, of a guinea pig, no cellular reaction other than the accumulation of macrophages takes place. (See page 241.) If, however, the catgut or charcoal be impregnated with some irritant substance such as turpentine, its injection is followed by a rapid and progressive accumulation of pus cells. Evidently, therefore, increased toxicity or irritant qualities of foreign particles determines more active leucocytic accumulation and phagocytosis.

In studying chemotaxis of phagocytes, Metalnikow[3] has noted the close relationship between this phenomenon and anaphylaxis. The introduction of a specific antigen into the

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[2]Aggressin is the name given to a hypothetical substance which is supposed to protect the bacterium from the action of leucocytes and antibodies.

[3]Metalnikow: Compt. rend. de la Soc. de Biol., May 21, 1921.

hypersensitive animal gives rise to an excessive inflammatory reaction. Guinea pigs and rabbits were rendered hypersensitive by repeated injections, and two weeks after the last injection small capillary tubes were introduced into the peritoneum and subcutaneous tissues. Some of the tubes were filled with specific antigen, while others contained an inert fluid. After ten to twenty-four hours the tubes were removed. Microscopic examination of the contents showed that very few leucocytes were present in the inert fluid, while those which contained antigen were packed with leucocytes. Further experiments showed that desensitization of the animals by means of injections of antigen inhibited leucocyte accumulation in the tubes.

Metalinkow has proved the positive chemotactic influence of antigen toward leucocytes in hypersensitive animals. He believes that in anaphylactic shock there is an accumulation of leucocytes at the focus of injection. There is a consequent leucopenia in the circulating blood.

If the tissues have been exposed to a previous parenteral introduction of the bacterial protein, they become hypersensitive to this protein, and there is present in the tissue and body fluids an antibody (first order antibody). Subsequent introduction of the bacterial protein is followed by an immediate reaction between this antibody and the bacterial antigen. In consequence of this reaction irritant products are developed; each individual bacterial cell thus becomes an irritant focus. As a result of this acquired irritative property the leucocytes are stimulated to move towards, and to ingest, the bacteria.

Experiments prove that it is not essential that bacteria be devitalized in order that they may be phagocytized by leucocytes. It is thus seen that even though, in consequence of either an inadequate concentration of proteolytic antibodies in the body fluids or special protective properties on the part of the bacterium, the body fluids alone are unable to destroy the microorganisms, nevertheless, a sufficient concentration of antibody may be present to indirectly accomplish destruction of the bacterium through the stimulation of cellular phagocytic activity.

Obviously, other things being equal, such bacteria as are protected by a surrounding capsule of either mucoid or waxy material (pneumococcus, streptococcus mucosus, B. leprae), are less likely to be acted upon by anaphylactic bodies present in the body fluids than are those which are not thus protected. In consequence we find that, *in vitro,* phagocytosis of such bacteria takes place but indifferently and that, clinically, such bacteria are allowed to proliferate and spread through the tissues before adequate vascular and cellular reactions take place.

Opsonin.—In 1902 Wright discovered that washed leucocytes ingested a larger number of bacteria from a suspension of the latter when in the presence of immune, as compared with normal, serum. For example, when, to a suspension of staphylococcus aureus, there is added a suspension of polymorphonuclear leucocytes, washed free from serum, few if any of the cocci are ingested by the cells. If to the mixture of cocci and leucocytes in salt solution, there is added a small quantity of fresh normal serum, each of the cells ingests a small number of bacteria. If, however, serum from an "immune" individual be employed, it is found that the average number of bacteria taken up by the cells is markedly increased.

As a result of his experiments Wright assumed the presence in the body fluids of a specific antibody as a result of the activity of which bacterial cell bodies were rendered more liable to phagocytosis by the leucocytes. He employed the word "opsonin" (opsono—I prepare food for) to designate this antibody.

Obviously, in the use of the word opsonin, Wright believed that the action of the antibody upon the bacterium resulted in the latter becoming more easily phagocytized by the leucocytes. In my opinion the action of "opsonin" upon antigen consists in so altering the bacterial cell protein that it acts as an irritant to the tissue cells. As has been previously pointed out, the majority of bacterial cells possess but little essential toxicity, or irritability, for normal tissues.

The number of bacteria ingested by polymorphonuclear leu-

cocytes in the presence of immune serum, as compared with the average number taken up by the same leucocytes when treated with normal serum, constitutes the opsonic index.

Wright found that, if too great a quantity of immune serum was added to a suspension of leucocytes and bacteria, phagocytosis of the latter was inhibited, as compared with the manifestation of phagocytosis exhibited when smaller quantities were employed. According to the author's hypothesis, such results are explained by the assumption of the presence in the serum of a sufficient amount of second order (tolerant) antibody to render nonirritating the product of the reaction between the first order antibody and antigen.

It is thus seen that, in the author's opinion, the opsonin of Wright is, in fact, identical with the anaphylactic or first order body; the degree of phagocytosis which occurs *in vitro* is dependent upon, either the absolute amount of first order body present, or the relative amount of second order body.

When an individual suffering from furunculosis is subjected to an injection of staphylococcus protein (vaccine) the following phenomena are noted. Within twelve hours, and continuing during the ensuing thirty-six hours or longer, the individual furuncles increase in size, the hyperemic zone becomes wider, and the lesions become more painful and tender. Small red papules may arise at points which had previously shown no evidence of infection. The patient may suffer from malaise. If the serum be examined, twenty-four hours after injection of the bacterial antigen, the opsonic index may be lower than before the administration of the vaccine. This is the condition described by Wright as the negative phase.

If Wright is correct in his conception that the negative phase is injurious to the injected individual, we must, if possible, guard against its occurrence. According to the author's conception of the negative phase, insofar as this refers to clinical manifestations, it indicates a stage of stimulation of morphologic tissue reaction consequent upon exhaustion of the second order (tolerant) antibodies. In the author's opinion, it is only insofar as the phenomena, which characterize the so-called

negative phase, are produced, that useful results are to be expected in the treatment of focal infections. It is the duty of the physician or surgeon, to so employ bacterial proteins (vaccines) that he is able to stimulate, and at the same time control and guide, the inflammatory reactions at the foci of infection throughout the body.

It is evident that it is only when a suitable dose of bacterioprotein is injected that a favorable result at the focus of infection is to be expected. If too small a dose of bacterioprotein be injected to depress, to any considerable degree, the second order body, no alteration in the inflammatory reaction at the bacterial focus takes place. In such an event, the injection is harmless, but except for whatever slight effect may be exerted upon the production of antibodies by the tissues by virtue of the added stimulus, no useful effect is accomplished. On the other hand, it is quite possible to so exhaust the second order body that a dangerously excessive inflammatory reaction may take place at the focus of infection. This untoward result may be due to the development of diffuse edema in a vital organ, such as the brain or the kidney, with consequent destruction of the individual, or, it may be in the nature of an excessive reaction in the sense of so increasing interstitial tension that circulation through the part may be interfered with and edema, with consequent liability to necrosis of tissue, ensue.

It is obvious, therefore, that extreme care must be taken in order to determine the proper dose of vaccine to be employed in treatment. In order that a suitable dose may be employed, it is necessary that the physician who attempts to alter the tissue reactions, should know the state of the tissues with reference to their hypersensitiveness and tolerance. It is also of the utmost importance, if it is the intention of the vaccine administrator to induce a severe reaction, that the possibility of focal accumulations of bacteria in vital organs, or in tissues in which interstitial edema is not well tolerated, be carefully excluded. In the treatment of infective lesions of the internal organs, but minimal increases in the hyperemic and cellular

reaction should be provoked. It must, moreover, be realized, that if multiple or extensive lesions be present, the multiple discharge of actively irritant substance, when the second order body is exhausted, may suffice to induce injuriously severe constitutional intoxication.

The author's observations have led him to believe that it is possible by means of the employment of the cutaneous, or better, the intradermic introduction of the bacterioprotein, and a close observation, during a forty-eight hour period following injection, of the reaction which occurs, to determine the relative proportion of first and second order bodies.

The protective property of leucocytes is exalted clinically by means of the induction of a leucocytosis by injections of certain substances, such as nuclein, collargol, olive oil, etc., which have been found to bring about an increase in the number of circulating leucocytes. Following the introduction of such materials, whether intravenously, intraperitoneally, subcutaneously, or per rectum, there ensues a period during which clinically there is a greater resistance against infection and the blood shows an increase in the number of leucocytes. Examination of the marrow, under such circumstances, demonstrates an increased myelogenous activity in this tissue. This phenomenon, which was first noted by Isaeff, is known as the *resistance period,* and has constituted one link in the chain of evidence giving to the leucocytes their place among the protective properties at the disposal of the body.

It may not be out of place to note at this point that the induction of reactions by means of the injection of peptone or partially autolyzed bacterial suspensions, and horse serum, owes its practical usefulness to a great extent to the stimulation of leucocytic production in this way.

Such methods of nonspecific protein therapy are also of practical value since their injection results in exhaustion of tolerant bodies and consequently the development of positively irritant properties on the part of bacterial foci in the body. Inflammatory—allergic—reactions with the exhibition of phagocytosis by the accumulated leucocytes may be thus stimulated.

By means of the injection of bacterioproteins or vaccines various results may be obtained, depending upon (1) the state of the individual injected; (2) the dose of bacterioprotein injected and (3) the interval allowed between injections. Thus any one of the following alterations in the hypersensitive state of the tissues may occur.

1. Hypersensitiveness may be induced if such be absent, or increased if already present.

2. Hypersensitive individuals may be desensitized.

3. Hypersensitiveness may be exalted and tolerance may be engendered by means of repeated injections.

4. In chronically infected individuals whose tolerance suffices to mask the hypersensitive state the immunizing bodies may be depressed so that their hypersensitiveness becomes more apparent and allergic reactions are stimulated.

5. Both sensitizing and tolerant bodies may be depressed to such a degree that all cellular and vascular reactions cease.

Relationship of Hypersensitiveness and Tolerance to the Phagocytic Reaction

It would appear that, although the body fluids are capable, under proper conditions, of digesting numerous foreign proteins including many bacteria, this method of bacterial destruction is not the one which is most efficacious and economical of body effort. Whenever possible the blood, or tissue, cells, more particularly the polymorphonuclear leucocytes, are employed in the reaction against insoluble particulate proteins.

The anaphylactic or allergic reaction plays an important part in stimulating phagocytosis. In the author's opinion this is explained in the following way. It is obvious that the phagocytic cells, more particularly the leucocytes, need some particular form of stimulus to induce them to migrate towards and to ingest, foreign substances. Now if we assume, as we have every reason to believe, that the majority of bacterial cells do not secrete, or excrete, any very potent essential toxin we may liken the inoculation of bacteria into nonsensitive animals to the introduction of plain catgut. Thus we find that

the intraperitoneal or other injection of dead tubercle or typhoid bacilli, staphylococci, etc., into normal animals or into man is not followed by vascular dilatation or leucocyte accumulation until after the lapse of a certain length of time, which corresponds to the period necessary for the anaphylactic state to develop. If, however, the animal employed has previously received a sensitizing dose of a like bacterial protein, we note that the phenomenon of allergy is demonstrated locally and, if a sufficient quantity be employed, systemic manifestations of intoxication are exhibited. The leucocytes from such an accumulation are found, moreover, to have ingested large numbers of microorganisms.

The explanation of these phenomena appears to be that as a result of the presence of specific first order antibodies (anaphylactin), in the body fluid of the sensitized animal, the injected bacterial cell is so acted upon by this substance that irritant products are liberated. In consequence the previously innocuous bacterial cell becomes at once an irritant focus, and so stimulates the leucocytes to activity. Phagocytosis of the bacteria and their subsequent complete proteolysis within the pus cell results. Granted that a sufficient number of bacteria be not ingested to bring about death of the cell itself this type of reaction is the most economical and most rapidly leads to the elimination of the invader.

In the ordinary course of chronic infections, such as tuberculosis, and staphylococcus infections, e.g., furunculosis, we have present in the serum not only antibodies capable of reacting with antigen so that substances are produced, (that is, not only is the individual sensitized to the tubercle bacilli or the staphylococcus as the case may be), but there is also present in its serum a substance which we have termed the second order antibody or "antianaphylatoxin." This substance is potent to neutralize and render innocuous to the tissues the irritant products arising from the reaction between antigen and first order antibody. If the proportion of the second order antibody to antigen be sufficient, the "anaphylatoxin," or allergic irritant product, is immediately neutral-

ized. Under such conditions cellular phagocytosis does not act as an irritant focus.

In such an event an increase in cellular activity can be stimulated by a depression or exhaustion of the second order antibody (tolerant antibody).

This point can, perhaps, be made more clear by a study of the changes which occur when tuberculin is injected subcutaneously into an individual suffering from a localized tuberculosis. We will assume that the individual is suffering from a small tuberculous collection in one of the cervical lymph nodes, but that he demonstrates no evidence of constitutional toxemia, such as increase of pulse rate or pyrexia. The subcutaneous introduction of .001 milligram of tuberculin (T.R.) is followed after a period of six hours or less with the onset of symptoms of constitutional toxemia, rapid pulse, elevation of temperature, headache, malaise and anorexia. Locally, at the point of injection there is slight swelling with a surrounding hyperemic zone of from 2-4 centimeters in diameter. This inflammatory area is tender and possibly painful. Focally in the neck the lymphnode becomes swollen and painful and may become the site of a definite collection of pus cells, i.e., abscesses may develop.

What changes have been brought about in this individual by the subcutaneous introduction of the tuberculin? We know from experimental evidence that a tuberculous individual contains in his serum first order antibodies (anaphylactin) or certain substances capable of so sensitizing guinea pigs that immediate anaphylaxis can be induced. Why does not this individual whose serum contains the sensitizing substance exhibit symptoms of tissue intoxication since there is present in his body a focus of tuberculoprotein? Two factors appear to me to be instrumental in permitting him to be free from fever and other symptoms of intoxication. In the first place, we know that under certain circumstances an animal loses its sensitiveness to small doses of protein antigen even though its serum contains a sensitizing body. This we assume to be due to the presence in the body of a second substance, which we

have termed the second order antibody (antianaphylatoxin), which has the property of detoxicating or rendering inert the product of the antigen-anaphylactin (first order antibody) reaction. This is sufficient to neutralize the comparatively small amount of irritant product which is liberated from the focus. A minimal reaction between antigen and antibody is likely to occur since the tuberculous nodule is, characteristically, poorly supplied with blood. The quantity of irritant body, by virtue of the fact that it is being constantly neutralized by the second order body (tolerant body) at the focal collection of tubercle bacilli, does not stimulate cellular reaction.

Upon the subcutaneous introduction of the tuberculo-protein there at once occurs a reaction between this antigen and the first order antibodies with the liberation of irritant products. If the amount of antigen introduced is sufficiently large, an excess of "anaphylatoxin" over available second order body will be present in the serum. As soon as this occurs the tuberculous focus assumes a definitely toxic property. As a result vascular and cellular activity at the point of infection is stimulated. There results, therefore, a triple manifestation of the reaction to a single injection of a bacterial protein in an infected individual, namely, focal inflammation at the site of infection, constitutional febrile reaction, and local inflammation at the site of injection.

Numerical values may be employed to explain the tissue changes that are exhibited. If the tissue of an individual suffering from tuberculous cervical lymphadenitis contain one thousand units of the first order body and fifty units of the second order body, and if the tuberculoprotein situated in the glands of the neck be so walled off by fibrous tissues that but twenty units of antigenic protein are brought in contact with the circulating body fluids, the patient manifests no evidence of constitutional intoxication, such as fever or malaise. The infected lymph nodes, moreover, are not tender, and show but little evidence of inflammation. If, into such an individual, a dose of tuberculoprotein, representing thirty-five units, be

injected subepidermically, the available second order bodies are exhausted and five units of anaphylatoxin are liberated from the artificial focus and the infected focus combined. If the injection has been made into the flexor surface of the forearm, the evidence of tissue irritation is manifested within a few hours by hyperemia and exudation of fluid into the tissues. In this way a red swollen nodule is produced. A similar reaction, though usually invisible, takes place in the glands of the neck.

If, instead of a dose of thirty-five units of tuberculoprotein, a dose of but ten units had been injected, the addition of the ten units to the twenty units which are being acted upon in the glands, would not have sufficed to exhaust or sufficiently depress the available second order bodies, to induce a reaction. In such an event no reaction occurs either at the site of injection, or in the focal lesions. On the other hand, if a dose of 600 units of protein antigen be injected, the amount of anaphylatoxin available, after exhaustion of the second order bodies, would be 570 units. We will assume that this number of units represents a relatively enormous dose of the irritant substance. In consequence, the forearm becomes much swollen, very red and painful, a lymphangitis is seen spreading up the arm and the glands in the axilla become tender. The affected glands in the neck undergo a similar reaction, pus cells are poured out, interstitial tension becomes extreme, and abscess formation and necrosis take place. At the same time, the patient suffers from hyperpyrexia and its concomitant exhaustion.

Obviously it is possible to administer so little bacterioprotein that no focal reaction is stimulated, or to inject so large an amount that an excessive reaction, both focally and constitutionally, is induced. It is likewise possible to so grade the dose of antigen introduced into the tissues that an adequate, but controlled, focal inflammation is stimulated. The aim of the clinical immunologist is to induce such an adequate reaction.

CHAPTER XVIII

PROTEIN-LYSIN IMMUNITY. ALEXIN (COMPLEMENT) AND SENSITIZING SUBSTANCE (AMBOCEPTOR)

In addition to the simple type of immunity reaction which is exemplified by the neutralization of toxin by antitoxin, a reaction occurs in which two serum bodies, acting together, destroy or otherwise alter the antigen. When certain particulate proteins, such as red blood cells or gram-negative bacilli, e.g., cholera vibrios, are treated with fresh immune serum, lysis of the antigen occurs. If the immune serum has been heated, such lysis does not take place; the addition of fresh normal serum, however, is followed by destruction of the antigen. The individual particles become obscure, and finally go into solution. It may also be proved that fresh normal serum is without effect on the antigen. By such a series of reactions, it was discovered that in order that lysis of a particulate antigen may be accomplished, two substances are necessary.

One of the serum bodies involved in this type of reaction is present in normal serum and is not affected in nature or quantity by immunity processes. This normal constituent of serum has been termed alexin (Bordet) or complement (Ehrlich).

The other antibody is specific in its action and is increased to a marked degree in the process of immunization. Since he conceived this substance to act as a link which unites antigen and complement, so that the latter may act upon the former, it was called by Ehrlich, amboceptor or midpiece. According to Bordet's conception the specific immune body renders antigen susceptible to the destructive action of alexin. He, therefore, applies to the amboceptor of Ehrlich, the term sensitizing substance (substance sensibilatrice). It is evident, from the use of the terms employed by these authors, that

according to Ehrlich's hypothesis, the antigen-amboceptor-complement reaction is accompanied by a building up, or synthesis, of the reacting substances, whereas Bordet believes that destruction, or dissolution, of antigen is accomplished by alexin when the former is rendered sensitive to the destructive effect of the latter through the action of the immune body.

Alexin or Complement.—The alexin is the most potent factor in the phenomenon of lysis; albeit, its activity cannot be exhibited unless the antigen has been rendered sensitive to its action as a result of a reaction with the specific immune body.

Alexin, or complement, is a normal constituent of serum and is not affected by processes of immunization. It is, moreover, remarkably constant in quantity, and is but little affected by differences in age, sex, etc., of individuals.

Alexin is a labile substance. It is destroyed by exposure to 56° for a period of one-half hour. It disappears, moreover, from serum in a comparatively short time, at ordinary temperatures. Its rate of diminution is more rapid at temperatures approaching that of body heat than in the ice chest. When exposed to temperatures of 17° to 27° C., a period of eight to twelve hours is sufficient to materially inhibit its activity. In the ice chest—3° to 5° C.—if stored in absolutely clean vessels, it usually maintains its activity practically unaltered for twenty-four hours, but should not be employed in serum reactions after such a period of time.

The actual cause of the disappearance of complement from unheated serum has not been definitely determined; it is probable, however, that it does not simply disappear, or that it is not destroyed by substances in the serum, but, rather, that it actually takes part in a nonspecific proteolysis which takes place following removal of blood from the body.

Fresh unheated serum which contains alexin (complement) is said to be "active." Destruction of the complementary body by means of heat (or other means) "inactivates" the serum.

The activity of serum may be maintained if the serum be dried, especially if desiccation be carried out *in vacuo* and at a low temperature. This fact also suggests that the deterioration in activating power in fluid serum is due to the occurrence of nonspecific proteotropic reactions.

The complementary activity of serum is destroyed by a number of simple chemical substances such as bichloride of mercury, silver nitrate, and by acids and alkalies. In the practical employment of complement in quantitative reactions it is essential that the glassware, rubber tubing, and other utensils employed, be perfectly clean.

Origin of Alexin.—There has existed for some time a difference of opinion with regard to the presence, or absence, of alexin, in the form in which we recognize it *in vitro*, in the circulating blood. A large number of observers, including Domeny,[1] Sweet,[2] Hewlett,[3] Lowit and Schwartz,[4] and Addis[5] and Dick, believe that their experiments prove the presence of complement as a normal constituent of plasma. Others, among whom are Gengou,[6] Hermann,[7] and myself,[8] have, as the result of their experiments, been led to adopt the view that complement, as usually studied, does not exist in natural plasma, but that it is developed as the result of subtle changes occurring in the blood following its removal from the body.

The reasons for such diverse opinions are that the methods adopted for the estimation of complement quantitatively, and also qualitatively, are liable to afford possibilities of error which are of the greatest importance.

It would be out of place in a work of this nature to enter into a prolonged discussion of the relative merits of these two points of view: since, however, I believe that the question is not without practical bearing upon several disease phenom-

[1]Domeny: Wien. klin. Wschnschr., 1902, xl, 105.
[2]Sweet: Centralbl. f. Bakteriol. I Orig., 1903, xxxiii, 208.
[3]Hewlett: Arch. f. exp. Path. u. Pharmakol., 1903, xlix, 307.
[4]Lowit and Schwartz: Ztschr. f. Heilkunde, 1903, xxiv, 205, 301.
[5]Addis: Jour. Infect. Dis., 1912, x, 200.
[6]Gengou: Ann. de l'Inst. Pasteur, 1901, xv, 232.
[7]Hermann: Bull. de l'Acad. Roy. de Med., 1904, 157.
[8]Gurd: Jour. Infect. Dis., 1912, ix, 225-234.

ena, a short résumé of my own opinions upon the subject is given. It is my belief that complement, as we are accustomed to consider it, does not exist as such in the circulating plasma, but that a body, which may be termed complementogen, is present in practically constant amounts. This complementogen is rendered active only as a result of the liberation of some substance, probably similar in effect to thrombokinase, which is produced by certain tissue cells,—in all probability the polymorphonuclear leucocyte. It is evident, if this hypothesis be correct, that as is the case with thrombinogen, an infinitely small quantity of the kinase is sufficient to render active a comparatively large amount of complementogen.

These views are based upon the facts observed,—that the amount of complement demonstrable in serum varies with the length of time and the temperature at which the blood is kept. This suggests that it is developed following removal of the serum from the body, the more so, since a temperature of 37.2°, or slightly higher, is more uniformly followed by a more rapid production of the substance than if a lower temperature be employed.[9]

There is no reason for believing, according to this hypothesis, that complement may not be produced, as occasion may arise, within the body, owing to the interaction *in vivo* of the two substances (complementogen and kinase).

It is not likely that the favorable results, occasionally obtained, in the treatment of infections by such nonspecific substances as horse serum, milk proteins, and perhaps the so-called Schaffer vaccines, are due in part to the stimulus afforded in this manner to the development of alexin in the body. It is noteworthy in this connection that the source of the kinetic body need not be from the same species as that from which the complementogen is derived.

Relationship of Complement to Leucoprotease.—In 1893 Buchner noted that aleuronat exudates, produced intrapleurally in rabbits and dogs, possessed a bactericidal value for *Bacillus coli communis* which exceeded the bactericidal power

[9]Gurd: Jour. Infect. Dis., 1912, ix, 225.

of the blood serum itself. Similar results were reported by Hahn, who employed B. typhosus.

Denys found that pleural exudates in rabbits, obtained by the injection of dead staphylococci and from which the cells were removed by centrifugalization, are more highly bactericidal for staphylococci than is the blood serum of the same animals. For a time, this increased bactericidal activity was believed to be due to the production of alexin on the part of the leucocytes themselves. It was, moreover, thought that alexin was identical with the proteolytic substance which may be extracted from washed leucocytes. That the presence of leucocytes in the exudates may, perhaps, result in the more rapid development of a maximum alexin content in the fluid is possible; that, however, alexin is identical with the leucocytic enzyme—leucoprotease (Opie)—is practically disproved.

"The enzyme of the polymorphonuclear leucocytes may be precipitated with alcohol and after drying may be preserved almost indefinitely. In the moist state it is destroyed by a temperature of from 70° to 75° C.; a lower temperature, 50° to 60° C., increases its activity. It acts in an alkaline or neutral medium, but is inhibited by acid. It is less active than trypsin *in vitro* and is not identical with alexin or complement." (Opie.[10])

These findings of Opie, with reference to thermostability of leucoprotease, are in accord with those of Schattenfroh,[11] Moxter,[12] Petterson,[13] and others.

A more recent study of this subject is that by Zinsser,[14] whose conclusions are abstracted in the following paragraph.

Extracts of normal rabbit leucocytes, both those obtained by queous extraction and those obtained by freezing in salt solution, have distinct bactericidal powers for pyogenic staphylococci and B. typhosus. There is considerable uniformity in the action of various lots of such extracts upon the same strain

[10]Opie: Jour. Exper. Med., 1905, vii, 316; 1906, viii, 410.
[11]Schattenfroh: Arch. f. Hyg., 1897, xxxi, xxxv.
[12]Moxter: Deutsch. med. Wchnschr., 1899, p. 687.
[13]Petterson: Centralbl. f. Bakteriol, 1905, i, No. 39; 1908, No. 46.
[14]Zinsser: Jour. Med. Research, 910, xxii, 397.

of microorganisms, and it is apparent that separate strains of the same species show no decided variation in their susceptibility to the bactericidal substances contained in the extracts. Immunization does not enhance the power of leucocytic substances. Fresh leucocytes have no power of reactivating inactive serum.

Multiplicity of Alexins.—Although the fact that there must exist a multiplicity of amboceptors is obvious, the question as to whether there is more than one type of complement is less easily answered. That the complementary bodies in different species of animals are not of identical structure with one another, or at least possess diverse affinities, is evident from the fact that the activating power of fresh sera from different species, e.g., the goat and guinea pig, upon another amboceptor containing serum,—e.g., the rabbit,—is not constant. Fresh goat serum contains a considerable quantity of complement as can be proved by means of experiments in which it is employed to activate hemolytic amboceptor derived from the goat. Goat serum is, however, of comparatively little potency in the reactivation of inactivated rabbit serum.

Although it is a fact that the complementary body derived from different animal species is variable in structure, there is no proof that one and the same serum contains more than one body of this nature. Experiments which have been interpreted as supporting the view of multiplicity of complement are all subject to other interpretation.

Sensitizing Substance (Amboceptor).—The specific substance as the result of whose presence in immune serum complement or alexin is bound to foreign cells, bacteria and heterologous proteins, has been termed by Ehrlich, the amboceptor. This name was chosen since Ehrlich believed that the substance acts by virtue of the formation of a link between antigen and complement.

Other observers have applied various terms to Ehrlich's amboceptor, each of which is suggestive of the activity of that body. Since it is the most important antisubstance produced during the process of immunization, it is commonly

called the immune body. Bordet, realizing that it attached itself to antigen and thus prepared the latter for the lytic action of alexin, calls it the sensitizing substance (substance sensibilatrice). Simple translation of the classic term amboceptor gives us "intermediate body" or "mid-piece."

The antibodies which determine the occurrence of such phenomena as cyto and bacteriolysis, phagocytosis, and anaphylaxis, are of the nature of amboceptors. The term amboceptor does not, therefore, indicate the nature of the end result of the activity of the substance, but simply the fact that we are dealing with a substance developed in tremendous excess during the process of immunization, but which requires the presence of alexin or complement in order that its action may be manifest.

Resistance of Amboceptor to Heat and Desiccation.—Amboceptors are relatively stable bodies. They resist a temperature of 60° C. for at least one hour, but with a distinct diminution in potency (about one half). They are quickly destroyed by a temperature of 75° to 80° C.

When serum containing specific amboceptors is preserved, under sterile precautions in the dark and at a low temperature, 5° to 8° C., the properties of the substance are maintained over a period of many months with but little deterioration.

Desiccation, *in vacuo,* does not injure the amboceptor content of serum and, when dried, the material may be preserved almost indefinitely without much diminution in strength.

Amboceptors consist of, or are closely related to, the globulins and may be procured in concentrated form by means of precipitation or "salting out" of the latter substances from sera.

Sensitizing Experiments.—If activated antisheep erythrocyte rabbit serum be added to a suspension of sheep's red blood cells and incubated for one hour, or placed for a longer period in the ice chest, subsequent centrifugalization of the mixture proves that the specific bodies have been removed from solution and that the cells have become sensitive to the action of

complement. In mixtures, therefore, of specific amboceptor and antigen, the latter absorbs or binds the former, even though no obvious change in the structure of the antigen occur.[15]

Reciprocal Activity of Complement and Amboceptor.—To a certain extent, the reaction between antigen, amboceptor and complement is one which can be accurately estimated quantitatively. Thus the proportions of each may be so determined that it can be stated that one unit of amboceptor plus one unit of antigen will require one unit of complement to produce complete lysis of the antigen (e.g., sheep's red blood cells). Three units of each of the three substances, likewise, result in a complete reaction. If, however, in place of one unit of complement and amboceptor, respectively, one half unit of either reagent be employed, it is found that the deficiency in amount of the one body can be compensated for by means of an increase in the quantity of the other serum used.

Multiplicity of Amboceptors.—It is evident from what has already been stated, with reference to the specificity of amboceptors, that the serum from one individual may contain an almost unlimited number of different amboceptors. It is, moreover, possible by means of absorption experiments to remove these separately from the serum. It must be noted, however, that the same phenomenon of group antibodies is noted with regard to amboceptors as is the case with other immune bodies, agglutinins, precipitins, etc.

It is noteworthy in this connection to refer to the fact that the continuous accumulation of experimental evidence bearing upon this subject indicates that although the various amboceptors—lysins, anaphylactins, etc.—are specific in their action, they are all very closely related to one another and that structurally they resemble one another very closely. It is not improbable that these various substances are, in fact, identical and that the fact that different names have been ap-

[15]If the antigen be in particulate form, agglutination may be exhibited; if in solution, precipitation may be obtained.

plied is due to the different forms of experiment which have been employed for their identification.[16]

Complement Fixation or Binding.—One of the most important, and most commonly employed, diagnostic serum reactions depends upon the fact, as first demonstrated by Bordet, in 1904, that sensitized antigen will bind with, or fix, complement if such be added, even though the amounts of serum employed be insufficient to bring about lysis of the antigen employed.

The usefulness of this reaction becomes evident, if by means of a hemolytic series minus complement, the presence or absence of free complement in a mixture can be demonstrated. Since this principle is so widely employed in the diagnosis of syphilis (Bordet-Wassermann reaction) at the present time, it is the subject of more extensive consideration in the next section.

Complement Binding Reactions

Bordet-Wassermann Reaction.—In 1904 Bordet and Gengou published from the Pasteur Institute in Paris, a method of serum examination whereby the presence of specific sensitizing bodies (amboceptors) in tested sera may be determined with very great accuracy.

This method consists in the application of principles, referred to in the last section, whereby the presence or absence of free alexin (complement) in a mixture of antigen, tested serum, and complement containing serum is discovered by testing the effect of the mixture, after incubation, upon sensitized red blood cells, or upon a suspension of red blood cells and a specific hemolytic amboceptor. That red blood cells are employed, with a specific hemolysin, as an indicator is merely a matter of convenience, such as the use of phenolphthalein, or litmus, in titrations for acidity.

Bordet's method of performing the reaction consists in the addition of inactivated serum to be tested, and fresh guinea pig serum (containing a known quantity of complement) to

[16]See Chapter XX. Identity of Antibodies.

a suspension, or extract, of the suspected microorganism. This mixture is incubated at 37° to 38° C. for one hour.[17] At the end of this time, a known quantity of sensitized erythrocytes (sheep) is added and the whole placed in the incubator for one hour.

If the tested serum contain specific amboceptors or sensitizers for the microorganism used, a union of antigen and amboceptor takes place and the complement present is absorbed or bound. The subsequent introduction of the sensitized blood corpuscles is not, therefore, followed by lysis.

If such specific antibodies are absent from the tested serum, the complement present is not bound or fixed and is, therefore, able to exert its lytic action upon the sensitized erythrocytes.

The principle underlying the reaction is simple, but in actual practice great care must be exercised, and considerable experience is necessary, if the results of complement binding reactions are to be relied upon.

The more important points which require attention and the most serious sources of experimental error include the following:

1. Since so many human sera contain small quantities of a multitude of proteotropic as well as lipotropic complement binding bodies, the presence of complement binding bodies can only be accepted as diagnostic of infection, if they be present in abnormal quantities.

The various reagents employed must accordingly be standardized not only qualitatively, but also quantitatively.

2. Since certain of the reacting bodies, at least, bear a reciprocal relationship to one another, they must be standardized against one another. At least two known factors must be at the command of the experimenter in order that the remaining materials may be standardized.

3. Although the normal complement content of guinea pig serum is remarkably constant in amount, it is very easily

[17]Complement is also bound, but more slowly, at lower temperatures. Indeed greater specificity is probably obtained if a temperature of 7° C. be employed.

destroyed if not properly preserved and if clean glassware be not used. It must always be titrated before use.

Before attempting to perform a test it is necessary that the potency of the hemolytic amboceptor be determined. Since fresh guinea pig serum, if procured and preserved in a constant manner, contains a very constant amount of complement and since the number of erythrocytes employed can be accurately determined, this is not difficult.

The unit quantities of tested serum have been determined experimentally for the different symptoms employed. Wassermann employed 0.2 c.c. of inactive patient's serum. If 0.2 c.c. of inactive patient's serum be accepted as a unit and 0.1 c.c. of normal fresh guinea pig serum as the amount of complement, the antigen must be so prepared, and used in such quantity, that the serum of known infected individuals consistently binds complement, in the presence of the antigen, and the serum from normal individuals gives regularly a negative reaction.

Bordet's technic for the identification of antibodies is employed in the diagnosis of disease and in the differentiation of antigen. In practice such reactions are used more especially for the purpose of determining the presence of antibodies to the gonococcus and the tubercle bacillus, as well as in the serum diagnosis of syphilis.

The serum diagnosis of syphilis since its description by Wassermann in 1906, has risen, comparatively rapidly, out of the class of experimental procedures. Its usefulness in diagnosis and also in the control of treatment has been established and the reaction has now taken a firm place among clinical laboratory aids. This method is known as the Bordet-Wassermann reaction.

Wassermann prepared a syphilitic antigen by shaking up chopped syphilitic liver mixed with sand in salt solution in the proportion of one to five to which one-half part of 5 per cent carbolic acid solution had been added. The precipitate is allowed to settle and the supernatant fluid employed as antigen.

Since Wassermann's original communication in 1906, of a method for the diagnosis of syphilis by means of the identification of specific bodies present in the serum of luetic individuals, many unnecessary changes and several important and valuable improvements in technic have been brought forward. Of the modifications suggested, and proved valuable, that of the more precise determination of the active principle of the antigen has been universally recognized. Soon after the original communications it was shown both by Wassermann himself, and by others (Levaditi and Landsteiner), that extracts of the spirochetes as prepared from syphilitic livers were not essential constituents, but that lipoid bodies capable of extraction, both from the liver and from other organs, preferably the human heart, were more potent. To Noguchi in particular, recognition is due for the demonstration of the rôle played by the phosphatid group of lipoids in the binding of complement in the presence of the so-called syphilitic antibodies.

CHAPTER XIX

AGGLUTININS AND PRECIPITINS

The serum of the immune animal exhibits certain properties which are being constantly made use of by the clinician, the bacteriologist, and the biologic chemist, in the diagnosis of disease, the recognition of proteins from different sources, and the differentiation of bacterial strains. Two of the phenomena are due to the presence of substances which are known respectively as agglutinins and precipitins.

Agglutinins

The first report of systematic research upon the subject of agglutinins was published in 1896 by Gruber and Durham.[1] It was noted by these observers that the serum of an animal which had received repeated injections of a suspension of a certain strain of bacteria, as for instance the *B. typhosus*, contains substances which so act upon suspensions of typhoid bacilli in normal salt solution that the individual bacterial cells come together to form clumps. Since the bacteria which are clumped in this manner are said to have agglutinated, the antibodies, as the result of whose presence the phenomenon occurs, are called agglutinins. A similar reaction occurs when nonbacterial particulate protein substances, such as red blood cells, are employed as antigen. The viability, or otherwise, of the bacteria in suspension does not affect the result of the experiment. If the bacteria belong to a motile species, they first of all lose their motility and subsequently clump.

The observations of Widal upon the development of the agglutination reaction in the course of typhoid fever placed this method of diagnosis upon a sound basis. In practice the

[1]Gruber and Durham: München. med. Wchnschr., 1896, pp. 213, 285.

method is universally used for the differential diagnosis of typhoid from similar fevers and is known as the Widal reaction.

Macroscopically, the clumps of bacteria become apparent after a greater length of time than if the suspension is examined under the microscope. If the suspension contain a sufficiently large number of microorganisms and the serum be sufficiently concentrated, the clumps become visible very quickly—in one or two minutes. This fact has been taken advantage of in the technic devised by Bass for the bedside diagnosis of typhoid fever. It should be noted, also, that if the bacterial suspension is shaken the cells are more intimately brought in contact with one another and agglutination consequently occurs more rapidly.

Normal sera contain very little agglutinin; the property of producing agglutination is, however, rapidly and extensively acquired during the process of immunization. Agglutinins appear within three to six days after injection and increase in quantity very rapidly. Whereas, normal sera rarely agglutinate bacterial cells (in suspensions containing 500 million microorganisms per cubic centimeter), when diluted in nine parts of salt solution, it is possible to induce the production of these antisubstances to such a degree that clumping of bacteria occurs even though only one part of serum be added to 100,000, or even 1,000,000 parts of salt solution, containing bacteria.

Group Agglutinins

The reaction of agglutination is a specific one but demonstrates, even more clearly than many other immunity reactions, the group relationships of many bacteria. Thus if an animal receives repeated doses of the *B. typhosus* its serum will be found to possess the property of agglutinating the colon bacillus, the dysentery bacillus and the paratyphoid microorganisms, although its agglutinating power, when treated with the homologous antigen, is exhibited in much greater dilutions.

That this phenomenon is due to the presence of group agglutinins in addition to specific antibodies is proved by the

fact that by a process of absorption it is possible to remove the group agglutinins and to leave specific bodies in the serum. If a serum, as, for instance, that against the typhoid bacillus in the above table, be treated successively with colon and typhoid bacilli, it is possible to cause agglutination to a limited extent of each type of bacillus. If the suspension of colon bacilli in salt solution plus serum be centrifugalized, and the bacteria thrown down and removed, it is found that the serum still maintains to a considerable degree its property of agglutinating the *B. typhosus.* Durham[2] has explained this

RELATIVE AGGLUTINATING POWER OF SERA FROM IMMUNE ANIMAL WHEN TREATED WITH HOMOLOGOUS AND HETEROLOGOUS TYPES OF BACTERIA

SERUM	B. COLI COMMUNIS	B. TYPHOSUS	B. DYSENTERIAE	B. PARATYPHOID A.
B. Typhosus	4,000	100,000	100	14,000
B. Coli	120,000	10,000	1,200	15,000
B. Dysenteriae	5,000	2,000	200,000	100
B. Paratyphoid a.	7,000	15,000	100	100,000

phenomenon of group agglutination by assuming that when an animal is inoculated with immunizing doses of typhoid bacilli there are produced agglutinins which may be designated, A, B, C, D, and E; if paratyphoid bacilli be used there develop agglutinins B, C, D, E, and F; in the same way B. coli induces the production of C, D, E, F, and G; B. dysenteriae D, E, F, G, and H, etc. The result is that when antityphoid serum is treated with an excess of colon bacilli they are removed from the serum group C, D, and E, whereas A and B remain intact. This theory, of course, is entirely hypothetical, but well explains the reactions as they actually occur.

Nature of Agglutinin Reaction

As already stated the manner in which agglutinins bring about agglutination and the reason for their production is so far not properly understood. It has been determined, however, that in order that clumping may take place, it is neces-

[2]Durham: Jour. Exper. Med., 1901, v, 353.

sary that the serum and cells be brought together in salt solution. It is supposed that the reaction represents a change in ionization of the bacterial cells. This results from a chemical (colloidal) change occasioned by the action of the antibodies upon the antigen. Bordet believes, and his opinion is very generally accepted, that as the result of a decrease in the solubility of certain components of the bacterial cell, there is an altered molecular attraction or tension between the objects themselves and the fluids which bathe them. This is evidenced by a diminution in surface tension which predisposes to agglutination. A similar phenomenon is exemplified when a suspension of clay which remains diffuse in distilled water clears as the result of clumping and sedimentation when salt is added.

The most usual method of estimating the agglutinating property of a serum or the agglutinability of a given strain of bacteria is by varying the dilution of the serum in the finished mixture. It has been pointed out, however, by Bass that, in order that this method may be reliable, it is necessary that the number of bacteria employed be accurately determined. Thus, it is found that, the larger the number of microorganisms, the smaller the amount of agglutinin which is available for action upon each individual bacterium. On the other hand, the further apart the individual cell units are, the more agglutinin is necessary to induce clumping. The technic recommended by Dreyer is based upon his appreciation of these facts. This author lays emphasis upon the necessity for employing suspensions that have been standardized by enumeration of the bacterial cells.

Precipitins

In 1897 Kraus[3] discovered that there develops in the serum of the immunized animal a substance which precipitates a cloudy deposit when added to a solution of the specific protein antigen by means of which the animal has been immunized. These substances are called precipitins.

[3]Kraus: Wien. klin. Wchnschr., 1897, p. 736.

Precipitins are produced against a large number of soluble protein substances—albumins and albumoses. The reaction is extremely specific, but results in the development of group precipitins, as is the case with other immune bodies.

Precipitins resemble other immune bodies in the manner and rate of their production. By means of fractional methods of precipitation of serum with ammonium sulphate it has been demonstrated that precipitins are closely allied to the globulins as are other antibodies.

As is the case with agglutinins, precipitins lose their power of inducing precipitation when heated to 60-70° for from five to ten minutes, nor can they be reactivated, by the addition of fresh normal serum (alexin).

The presence of alexin is not necessary for precipitation to take place. Mixtures of precipitin and antigen, on the other hand, do possess the property of removing complement, if such be added, from the mixture.

The fact that, by the action of complement upon precipitated proteins, there is developed a soluble toxic substance has received attention elsewhere (Anaphylatoxins, see page 130).

Precipitins are capable of absorption in the same manner as agglutinins. This might well be expected in view of this probable identity of the two substances.

The most commonly employed practical use of the precipitin reaction is that of the identification and differentiation of proteins, as for instance, in criminological investigations. In order to discover the origin of bloodstains, the material from the stain is dissolved in salt solution, and treated in series with sera from animals (rabbits) immunized respectively against sera from several sources—horse, dog, swine, cattle, man, etc. If the stain be due to deposit of dog's blood, no precipitation, in high dilutions, occurs, unless the questionable material is treated with serum from a rabbit which has been immunized by the repeated injections of dog's blood.

CHAPTER XX

RELATIONSHIP OF ANAPHYLACTIN TO OTHER IMMUNE BODIES

Identity of All First Order Antibodies

The properties of neutralization of toxins, and lysis of proteins, which are assumed by the body fluids during the process of immunization, are due to the production by certain tissue cells of antibodies which are discharged into the body fluids. There are two main types of antibody reaction: (1) toxin-antitoxin reactions; and (2) proteolysis. The second type is again divided into two orders: (1) anaphylactic (first order) and (2) tolerant (second order). The reaction between toxin and antitoxin is a simple one: insofar as we know at present, the thermostable immune body—antitoxin—reacts with, and neutralizes, its antigen—toxin—without the help of any complementary body such as alexin. The antigenic substances which take part in such reactions are the true toxins of bacteria, snake venoms, enzymes, and numerous poisonous vegetable proteins. "It is to be noted that these active substances are all similar to one another in being classed as large colloidal aggregates resembling proteins, but not yet identified as proteins" (Wells).

The second type of immunity reaction is that in which the tissues produce antibodies against foreign proteins, whether essentially toxic or nontoxic, or whether soluble (serum or egg albumen) or particulate (bacteria, red blood corpuscles, tissue cells). In this type of reaction "we deal with processes that tend to alter the colloidal state of the foreign proteins by making them larger aggregates (precipitation, agglutination) or smaller aggregates (proteolysis, hemolysis, bacteriolysis, cytolysis), and in each case the reaction consists of two separate steps, sensitization and reaction." (Wells.)

The author believes that, in addition to the anaphylactic (first order) antibody, the tissues produce, in order to com-

plete proteolysis, a second order antibody, the function of which is to supplement the proteoclastic activity of the first order antibody, and thus to render nonirritating the product of the first order antibody-antigen reaction. The state of the individual or animal whose tissues contain the second order antibody is described in this volume as that of tolerance.

Although the subject is not yet finally settled by direct evidence, there are many observations which indicate that precipitin, agglutinin, and sensitizing antibody, as well as opsonin, run parallel in their content in immune serum. Experiments which support this point of view have been carried out by Friedberger, Doerr and Russ, Wells, Coca, and Weil. There is much to support the conception referred to by Zinsser as the "unitarian theory," which accepts the probable identity of the various immune bodies. The names applied to them, namely, agglutinin, precipitin, opsonin, sensitizing body (anaphylactic antibody) and complement-binding body, depend upon the nature of the phenomena which occur under varying conditions of experiment for their identification, rather than upon essential differences in the nature of the antibody responsible for the phenomena produced. In this volume the author has employed the expression "first order antibody" to designate this immune body.

At first sight the hypersensitiveness, which characterizes anaphylaxis, suggests that we are dealing with a phenomenon which is the very antithesis of immune body production. Upon further study, however, we find that, not only are the bodies which are responsible for its development subject to the same laws, insofar as these are known, as other immune bodies, but we are led to realize that the reaction, paradoxical as it may seem, is in reality a protective one.

The presence of specific antibodies free in the serum of sensitized and immunized guinea pigs is indicated by the following facts: (1) passive transference; (2) the prevention of passive anaphylaxis by the saturation of the anaphylactic serum with antigen; and (3) only by the assumption of specific antibodies in the blood can we satisfactorily account for the production of immediate anaphylactic shock in normal guinea

pigs by the injection of a mixture of antigen and sensitive serum.

As with other immune bodies, for example, precipitins and agglutinins, we find that a definite period of time (incubation period) must elapse before the amount of anaphylactin present in the blood reaches its maximum. Furthermore, it is noted that, once the production of the anaphylactic substance has been stimulated, it may be demonstrated in the blood for a longer or shorter space of time, up to seven or ten years or longer.

If the serum from a rabbit, which has been treated by repeated injections of sheep's red blood cells, be added in suitable quantity to a salt solution suspension of sheep's red blood cells, one or other of two effects will be noted. The difference in the results obtained depends upon whether or not the rabbit's serum has been exposed to the effect of heat. If the serum has not been heated, its action upon the red blood cells is exhibited by lysis of the latter. The cloudy suspension becomes clear, and pink in color, in consequence of the destruction of the cell bodies and solution of their hemoglobin content.

If, on the other hand, the serum has been inactivated by exposure to a temperature of 56° C., no alteration in the color of the mixture occurs. The cells maintain their identity, so that a suspension of cells in salt solution remains. That the cells are not unaffected is, however, shown by the attraction of cells to one another, so that clumping of cells—agglutination—takes place. If the cells be washed, so as to free them from the rabbit's serum, the fact that they have been altered, while in contact with the immune serum, is easily proved by one or other of the following experiments.

When a small quantity of normal fresh washed rabbit's or guinea pig's serum is added to a suspension of the washed sensitized erythrocytes in salt solution, lysis of the cells takes place. Again, if the sensitized cells be injected into a normal guinea pig, the animal shows symptoms of intoxication similar to those exhibited when the hypersensitive animal is "shocked" (Friedeman). These experiments indicate that the

substance responsible for the production of the anaphylactic phenomenon requires for an exhibition of its activity the presence of alexin (complement); also, that it is possible for it to bind with its specific antigen *in vitro,* even though no alexin be present.

That the content of precipitin and sensitizing antibody runs parallel in immune sera has been noted by several observers (Friedberger, Doerr and Russ). Wells has observed that precipitins appear at the same time as the capacity to confer passive sensitization (to anaphylactic shock). Both Weil and Coca performed experiments that appear to prove the identity of precipitin and sensitizing (anaphylactic) antibody. Friedberger's experiments in the production of "anaphylatoxin" through the action of alexin containing serum upon sensitized protein indicate that the complement binding antibody and anaphylactin are identical.[1]

Precipitation and agglutination are phenomena which may be induced by the treatment of antigen with heated serum. In other words, the thermolabile constituent of normal serum, known as alexin or complement, is not necessary for the exhibition of the phenomena of precipitation or agglutination. On the other hand, the lytic phenomena are dependent upon a reaction between the specific thermostable immune substances and antigenic protein, in consequence of which the later is rendered susceptible to the lytic activity of alexin. The end product of the reaction between first order antibody-antigen and alexin (complement) acts as an irritant which, especially in the case of antigens in particulate form, stimulates phagocytic cells to functioning activity (see opsonin). In this event complete dissolution of the antigenic molecule is accomplished within the cell cytoplasm by the action of enzymes (e.g., leucoprotease).

In Zinsser's opinion the relationship of the various anti-

[1]It should be mentioned in this connection that Friedberger's view, that the "anaphylatoxin" which is produced in this way is, in fact, the same substance which is responsible for the manifestations of anaphylaxis, has not been universally accepted by immunologists. Wells points out that "although many facts appear to support this hypothesis, there are others that do not harmonize with it, notably the lack of constant quantitative relations between the different reactions produced by the same immune serum, and as yet it is neither established nor completely disproved."

bodies to one another is "that much reasonable evidence points to the fact that the so-called precipitins are in truth protein-sensitizers, in structure and function identical with the sensitizers, or amboceptors, of cytolytic processes." The fact that precipitation occurs, when these antibodies are added to the homologous dissolved antigen, is merely a secondary colloidal phenomenon. Antigen and antibody react, forming a complex which is then amenable to the action of alexin. Since they are colloidal in nature, if they are mixed under suitable quantitative and other conditions which favor flocculation, they precipitate. This point of view, which identifies the so-called precipitins with the protein sensitizers or albuminolysins, was first hypothetically suggested by Gengou. It leads necessarily to the conception that in cytolysis as well as in proteolysis, in fact in all reactions in which antigen is sensitized to the action of alexin, there is functionally but one variety of antibody—the sensitizer, precipitation and agglutination being incidental physical phenomena and not dependent upon special antibodies as heretofore supposed. In this sense, then, the "precipitins" or albuminolysins may be regarded as identical with the anaphylactic antibody. (Zinsser.)

The anaphylactic antibody is thermostable and is not destroyed by a temperature of from 50° to 58° C. for one hour (Lewis, Anderson and Frost). In this respect this antibody simulates the immune bodies which are identified by other methods.

Experiments prove that the reaction between anaphylactin and protein is similar to that between amboceptor, sensitizing substance (Bordet) and antigen, inasmuch as alexin must apparently be present in order that the reaction be brought about.

The phenomenon of absorption (Bordet), which is noted as a characteristic of other immune bodies, also occurs in the interaction of antigen and anaphylactin. If to 4 c.c. of sensitive guinea pig serum .01 c.c. of the protein antigen be added, experiments prove that the serum has lost its property of transferring the hypersensitive state. Similarly, the injection of a small dose is sufficient to render an animal refractory, even though practically no symptoms have been elicited.

CHAPTER XXI

HEMOCELLULAR REACTION. THE LEUCOCYTE COUNT IN DIAGNOSIS AND PROGNOSIS

As the result of any alteration in the degree of stimulation of leucocyte activity, there occur characteristic changes in the number of circulating white blood cells. According, also, to the nature of the stimulus, the increase, or decrease, of cells is found to be confined to one or more special types. The recognition of certain broad principles underlying these variations has proved to be of great clinical usefulness, since by means of the simple procedure of counting the total number of cells in a given quantity of blood and estimating the proportion of each of the several types, it is not infrequently possible to diagnose with remarkable accuracy the nature of a disturbance in hidden parts of the body.

The value of leucocyte counts in infectious diseases depends upon the fact that, when leucocytic accumulation about an infected focus is stimulated, there occurs a corresponding increase in the number and type of cells discharged from the myelogenous tissues into the blood. Bacterial invasion by such microorganisms as the staphylococcus aureus, B. coli, B. pyocyaneus, pneumococcus, and streptococcus are usually met by focal accumulations of polymorphonuclear leucocytes. The reaction to these infections is, likewise, accompanied by an increase in the number of polymorphonuclear leucocytes in the blood. Similarly, certain infections, such as pertussis and frequently also tuberculosis, call forth an increase in the number of lymphocytes with little or no change in the number of other cells. Again the presence in the gastrointestinal canal of parasites and certain skin affections stimulate a more active manufacture and discharge from the bone marrow into the circulating blood of eosinophiles.

It is thus seen that, in a broad way, the nature of an infection is indicated by a study of the type or cell, which has been stimulated to appear in the blood stream in increased numbers.

In the diagnosis of the nature and extent of acute inflammatory reactions, in which the process cannot be visually demonstrated, there is no single source of evidence so useful as that derived from the examination of the blood. In order, however, that correct inferences as to the nature of the infective process may be made, it is necessary that adequate clinical data, such as the age of the individual, duration of the disease, record of pulse and temperature and results of careful physical examination should be studied in relationship to the blood findings. The total leucocyte count by itself is of less value than the differential count. For diagnostic purposes, moreover, it is essential that both total and differential counts be obtained.

Physiologic Variations in Circulating Leucocytes

The total count, and to a less extent, the differential count is influenced by several physiologic states, more particularly digestion, pregnancy, parturition, violent exercise and massage in each of which there is an increase in the number of white cells.

The total count indicates, therefore, the individual's power of reaction, either in response to natural physiologic demands, or against infecting microorganism. "The relative polymorphonuclear leucocyte count is an index of the degree of, or severity of, an infection." (Hewitt.[2])

Quoting again from Hewitt: "If we have a relative polymorphonuclear count ranging between 75 per cent, and 80 per cent, infection is usually found; if above 85 per cent, infection is almost invariably encountered and this regardless of the total number of leucocytes."

The information usually desired by the surgeon when making a leucocyte count, comprises the following:

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[2]Hewitt: Annals of Surg., 1911, liv, 721.

1. The resisting power of the individual, thus indicating the reserve force at the command of the body.

2. The severity of the infection, or the virulence of the infecting microorganism.

3. The localization, or diffusion, of the infecting agent and the accompanying reaction.

Gibson[3] suggests the use of a chart[4] whereby the disproportion of total count and relative polymorphonuclear count may be clearly shown. His chart is based upon the assumption that 10,000 white blood cells per cubic millimeter is the maximum normal count, and that 75 per cent is the highest percentage of polymorphonuclear leucocytes which should be considered normal. "In inflammatory lesions which are well borne, the polymorphonuclear cells are increased one per cent above 75 per cent for every 1,000 total leucocytes above 10,000." By estimating the nature of infection and resistance according to this hypothesis, valuable information may be obtained.

If an infection is well resisted, i.e., the reactive reserve force is adequate to control, for the time being at least, the spread of the invading bacteria,—the chart shows a parallel line, or one in which the decline is towards the right. If the line be level and placed high, it indicates a severe infection, but accompanied by a correspondingly marked or adequate reaction.

In those cases in which clinical symptoms and signs indicate an important affection, the presence of a line ascending toward the right suggests a bad prognosis. This is the observation commonly derived from blood counting in cases of diffuse or general peritonitis, and demands as a rule, immediate operative interference, and the employment of other measures indicated in such cases.

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[3]Gibson: Annals of Surg., 1906, xxxiv, 485.

[4]Gurd: New Orleans Med. and Surg. Jour., 1914, lxvii, 67.

CHAPTER XXII

FOCAL VASCULAR AND CELLULAR REACTION TO IRRITANTS. INFLAMMATION

As a result of the presence in the animal tissues of irritant substances, there occur morphologic changes which have for their purpose the control and elimination of the injurious agent. These changes consist primarily in dilatation of blood vessels in the part. This applies to the capillaries as well as to the arterioles and the venules. Inasmuch as, under normal circumstances, only a part of the total number of capillaries function as blood channels, their dilatation results in the presence of an increased amount of blood in the part, although other factors may interfere with an adequate circulation of blood through the part. If sufficient stimulation be present, new blood vessels (capillaries) form, as the result of budding, from the walls of existing capillaries. The second phenomenon which occurs, consists of an exudation of blood fluid from the vessels into the surrounding tissues. The third essential phenomenon of inflammation is manifested by an accumulation in the interstitial spaces of an increased number of cells. Clinically, this hyperemia, accumulation of interstitial fluid, and focal cellular increase, is manifested by swelling (edema), redness, and perhaps pus accumulation. It is this local reaction upon the part of the tissues against injurious substances which is known as inflammation.

Inflammation is the process by means of which cells and serum accumulate about an injurious substance and tend to remove and destroy it; this process does not include the regenerative changes which replace injured tissue by newly formed parenchymatous elements or by new interstitial tissue (Opie).

Practically all foreign substances, when introduced parenterally into the tissues, cause an accumulation of more or less serum, and attract a larger or smaller number of new cells to

the part. The process is in many respects similar to that which occurs normally in the alimentary tract; indeed, in many of the lower animals (protozoa and infusoria), the method by which the cells are normally nourished, is similar to that which occurs in man and in the higher types of mammals only during the inflammatory process.

In general the purpose of the inflammatory reaction is to neutralize or dilute toxic soluble substances, to liquefy and absorb digestible substances, and to engulf by cellular activity such materials as cannot be rendered soluble by the action of the fluids of the body alone.

It must be remembered by the practicing surgeon that inflammation is, in purpose, conservative and protective in nature, and that any operative or other interference must be undertaken only after careful consideration of the effect of such procedure upon the reacting process.

It is the duty of the surgeon to see to it that, insofar as the effects of inflammation are useful, they should be conserved, and, if possible, stimulated to increased activity, but also to recognize that, when carried to extremes, the inflammatory reaction may not only be ineffective, but may in itself do harm. Steps should be taken to prevent, insofar as possible, such injurious effects. Inasmuch, moreover, as the reparative reaction may also be followed by serious disability, the necessity of excessive tissue fibrosis should be prevented by early interference, having as its aim the elimination of the necessity of organization by granulation and consequent fibrosis.

Since inflammation consists in the local accumulation of serum and cells, it is essential, if the reaction is to be understood, that (1) the nature of the essential qualities of the serum, and (2) the nature and method of action of the cells taking part, be appreciated.

The manner of accumulation of both cells and serum, moreover, is of the greatest importance, since it is in the surgeon's power,[5] by means of incision, immobilization, hot and cold

[5]See Chapter XXVI on the Therapeutic Guidance of the Acute Inflammatory Reaction.

applications, active and passive hyperemia, and the employment of vaccines, to influence to a considerable extent the factors which determine an adequate hyperemia, and serum and cellular exudate.

Nature and Qualities of Serum

The focal accumulation of body fluid, which takes place in inflammatory reactions, is valuable inasmuch as the blood and lymph contain, in solution, substances which are capable of causing alterations in the composition of complex proteins, neutralizing toxic materials, and of stimulating phagocytosis of insoluble bodies.

In addition to the foregoing specific resisting properties of body fluid, the increased circulation of blood and interstitial fluid (lymph), through the inflamed focus, aids in dilution of toxic substances, whether these be due to bacterial activity or are the result of breaking down of tissue proteins. Increased blood circulation through the part is automatically accompanied by an increased nutrition of the fixed tissue cells; also, there is brought to the part a larger number of polymorphonuclear leucocytes than under normal conditions. As a result of the exudation of plasma from the vessels into the interstitial tissues, or into potential cavities, fibrin is deposited.

Occasionally the fibrin deposited in this way serves a positively useful purpose, as, for instance, in sealing openings in the abdominal viscera, and in delimiting the spread of infection in serous and synovial cavities. The presence of fibrin, on the other hand, acts as a direct stimulus to fibroblastic proliferation, and thus to the development of permanent adhesions in joint surfaces, between coils of intestine, or about the foramina at the base of the brain, thus leading to their occlusion.

The fluid constituent of inflammatory exudate is usually of a higher specific gravity, and of a higher protein content, than is the blood plasma. This fact must be assumed to be due to some selective action on the part of the vessel endothelia.

Phenomena of Inflammation

Accumulation of Serum.—Although the inflammatory reaction consists, in addition to hyperemia, in a local accumulation of cells and serum, the amount of serum and the number and type of cells varies according to the nature of the irritant and its concentration.

The purpose, therefore, of the reaction which is manifested by the development of hyperemia and interstitial edema, is the dilution, neutralization, and lysis, of the irritant. It is evident that, if the maximum benefit is to accrue from the action of the serum antibodies, not only is it essential that an increased amount of fluid find entrance to the zone of irritated tissue, but that removal of exhausted fluid and its replacement by fresh plasma take place at as short intervals as possible. In order that this may be accomplished, there occurs: (1) an increase in the blood supply to the part; (2) an increase in the amount of fluid passing from the arterioles into the extravascular tissues; and (3) the rate of removal of fluid from the inflammatory focus by means of the veins and lymphatics becomes more rapid.

In order that an increased amount of fluid may be discharged into the tissues, the afferent vessels (arterioles) of the part dilate, many capillaries which normally do not contain blood commence to function, and often new blood vessels develop by a process of budding.

Even though there be an increased local afferent blood supply and a greater tendency than normal exhibited for the passage of fluid from the vessels, the possibility of continued and rapid circulation of blood through the vessels and continued exudation of fluid will depend upon (1) an efficient drainage of the inactivated fluid from the part by means of the veins and lymphatics, and (2) the capacity of the tissues to expand sufficiently to accommodate an increased amount of fluid without raising the extravascular pressure above that in the venules and arterioles. In other words, the continuation of the exudative process depends largely upon the relation of intravascular to interstitial pressure.

In the presence of excessive exudate in the interstitial tissues, with consequent increase in extravascular tension, very little blood is permitted to flow through the part. As pointed out by Opie, this fact helps to explain the obvious truth that accumulations of fluid in the subcutaneous tissues, in response to irritation, are quickly self-limited, whereas the same substances, which cause such accumulations, may be able to induce the exudation of an immense amount of serous fluid when introduced into a serous or synovial cavity.

Clinically, the presence of interstitial fluid is evidenced by a swelling which characteristically ''pits'' upon pressure. Occasionally, however, this phenomenon is not noted, and there occurs a condition which is often referred to as a brawny swelling or induration. In certain instances this may be due to the presence of an excessive fibrosis. Recent experiments (Fischer), however, suggest that under certain circumstances of relative acidity of the tissues, the collagenous fibers of the connective tissue, which are composed of a colloidal protein material, become ''fixed'' through the absorption of fluid in a manner similar to that of agar-agar in the preparation of media.

As may be easily understood, the stretching of the blood vessel wall and the injurious action of the irritant substance may occasionally result in the passage through it not only of the blood plasma, but also of the blood cells. Clinically and experimentally, we note that such hemorrhagic phenomena are found in the presence of the more potent irritants, e.g., in infections due to the streptococcus hemolyticus or the bacillus aerogenes capsulatus—since these agents appear to injure directly the vessel walls.

Nature and Origin of Cells Which Take Part in the Inflammatory Process

Polymorphonuclear Leucocytes.—The most active and apparently most powerful cells which take part in the defense of the tissues against foreign irritants, more especially bacteria, are the neutrophile polymorphonuclear leucocytes of the blood.

These cells are produced in the bone marrow, discharged thence into the blood stream, and attack irritants situated in the interstitial tissues. Under the microscope they may be seen leaving the capillaries between the endothelial cells by a process known as diapedesis.

Once the polymorphonuclear leucocyte has been discharged into the blood stream from the bone marrow, there is no proof that it retains the power of reproduction, so that in inflammatory foci the accumulation of cells is believed to be possible only by the passage of new cells from the blood stream into the extravascular tissues. The polymorphonuclear leucocyte does not appear to take part in reparative processes.

That the polymorphonuclear leucocytes have an inherent bactericidal power is proved by experiments first performed by Powlowski, namely, that sterile inflammation accompanied by purulent exudate is highly protective against subsequent infection. This is in all probability explanatory of the results which follow injections of sterile irritants, such as formalized glycerine into the knee joint, in the treatment of chronic and subacute infections of synovial cavities.

Not only does the polymorphonuclear leucocyte employ its leucoprotease content to digest ingested material, but it discharges a certain amount of this substance into the tissues about itself. Under ordinary circumstances this excreted ferment is inactivated by an antiferment which is present in normal body fluid. If the number of leucocytes be relatively great, as compared with the amount of body fluid (serum), there is exhibited an excess of leucoprotease. Such a purulent material affects not only irritant protein substances but even living tissue may be injured. It is, in part, on account of the presence of this enzyme, whose nature has been studied particularly by Opie, that the phenomenon of burrowing on the part of pus collections occurs.

It does not appear, however, that the enzyme constituent of pus is the chief cause for the spread of suppuration. Tension in the tissues, as the result of increased extravascular fluid, is the most obvious reason for the phenomenon. This is clearly

exemplified by the prompt subsidence of a spreading lymphangitis of the forearm and arm, when the focal lesion in the finger is adequately incised. Another important cause, and one which is not infrequently overlooked in surgical procedure, is the force of gravity in determining the spread of pus. In the author's opinion, this is one of the chief objections to the employment of the extension-suspension method of treating infected compound fractures of the extremities. I have frequently seen burrowing abscesses between the muscle sheaths in the thigh, in cases of gunshot fractures of the femur, which had been spreading up the thigh and even into the buttock while the limb was elevated, quickly cease to spread and the purulent process subside, when, instead of elevation of the foot, the head of the bed was raised and the foot placed at a lower level than the hip joint.

Residual pus in the tissues acts as a foreign body and, in addition, as a pabulum for growth of microorganisms. It is a primary principle of surgery that sterilization of the tissues, whether the body's resisting forces are alone relied upon, or whether these properties are augmented by the employment of adjuvants, such as Dakin's solution, according to Carrel's technic, B.I.P.P., flavine, or other method, is impossible so long as foreign bodies are present which may injure the tissues. It is of no practical importance whether such foreign bodies consist of shell fragments, rubber tubes, necrotic fascia, sequestra, or devitalized body fluids (pus). Protected by the injurious effect of such foreign bodies upon the tissue, bacteria are enabled to proliferate, and from the focus thus produced to invade the surrounding tissue.

CHAPTER XXIII

CLASSIFICATION OF INFLAMMATORY REACTIONS

Inflammatory reactions have been for many years divided into three groups—acute, chronic, and granulomatous, and since such a classification of individual lesions is usually possible and likewise profitable, such a method of division has been employed in this chapter.

In *acute inflammation,* serum and polymorphonuclear leucocytes accumulate at the site of irritation. It is the method employed by the tissues in their attack upon most bacteria, more especially the so-called pyogenic cocci, although it must be remembered that, granted the irritative property of a sterile body equal to that of pyogenic bacteria, the character and sequence of the inflammatory events are identical, except insofar as the absence of a proliferating irritant influences the spread and continuation of the reaction.

The use of the terms "acute" and "chronic" is based upon the fact that the former is usually more or less fulminant and short lived. Chronic inflammatory lesions, on the other hand, are, at their maximum, relatively mild in clinical manifestations and are usually prolonged. It should be noted that the acute reaction is very frequently followed by a chronic process.

Chronic inflammatory reactions are those in which the most prominent part is taken by cells of the lymphoid group, and by the so-called macrophages, to the relative exclusion of the polymorphonuclear leucocytes. There is usually a minimal accumulation of interstitial serum. This is the type of reaction which is found in the presence of mildly toxic insoluble substances, notably those resulting from the destruction of body tissue *in situ,* and usually accompanies reparative changes.

Certain types of bacteria which possess but little essential toxicity, whose rate of growth is slow, and which, owing to a thickened ectoplasm or capsule protection, are but slowly

acted upon by serum antibodies, also induce such a reaction, which, since it often resembles granulation tissue, is known as the *granulomatous reaction.* Since many of the cells which take part in the reaction of these types multiply *in situ* at the focus of accumulation, this type of reaction is also called *proliferative,* in contradistinction to the exudative (acute inflammatory) lesion.

Acute Inflammation

Accumulation of Polymorphonuclear Leucocytes.—It is found in studying inflammatory processes that, if the foreign substances present in the tissues be sufficiently irritating, the polymorphonuclear leucocytes are the cells which play the major part. A study of the living tissue when subjected to irritation shows that the following changes are exhibited. Lister in 1855 was the first to record the vascular phenomena which characterize the reaction of the tissues to irritants. Coincident with the dilatation of the blood vessels there is a slowing of the blood stream; leucocytes collect upon the inner wall of the vessel (margination of leucocytes).

The leucocytes then commence to insinuate themselves between the endothelial cells, and pass by active ameboid movement—diapedesis—through the vessel wall and toward the irritant substance. It frequently happens that large numbers of cells are sacrificed, and, if the process continues sufficiently long, a collection of creamy fluid material accumulates.

Surrounding a purulent focus there is usually present a wall composed of new blood vessels and edematous tissue, in the meshes of which there are embedded numerous cells, chiefly polymorphonuclear leucocytes. This delimiting zone is known as the pyogenic membrane.

The process whereby pus accumulation takes place is known as suppuration. Pus is composed of varying proportions of the following substances:

(a) Foreign bodies—usually bacteria and their derivatives (e.g., toxins, protein split products).

(b) Fixed tissue debris—necrosis of tissue, due to ischemia and caused by the toxic action of bacteria.

(c) Serum—from which the original antibodies have been largely exhausted, and to which leucoprotease has been added.

(d) Polymorphonuclear leucocytes—for the most part dead or much injured.

The fluid property of pus is due to the presence of serum exudate from the vessels and the results of cytolysis. In this fluid is suspended larger or smaller numbers of cells, and a variable quantity of colloidal substances. Its consistency depends upon the ratio of fluid to cells and colloid. The color of the purulent fluid depends upon the color of the cells, and the presence or absence of colored products of bacterial activity. As a rule, staphylococcic infections result in the accumulation of a relatively larger number of polymorphonuclear leucocytes, and, since these organisms produce but little coloring matter, the typical staphylococcic pus is of a creamy or somewhat thicker consistency, and of a slightly greenish-grey or creamy color. Characteristically, also, purulent accumulation in staphylococcic infection is of a pasty odor. Since, as a rule, reaction to streptococci is usually less rapidly effective, and a limited pyogenic membrane is less constantly developed, there is a relatively larger quantity of serum than in the purulent fluid consequent upon infection by the staphylococcus aureus. Also, since the toxins of the streptococcus, and its variants, are more prone to lead to severe injury to the vascular endothelium with consequent extravasation of erythrocytes, pus from such lesions is characteristically more fluid and is likely to be blood-stained.

Organisms such as the B. pyocyaneus, which produce a comparatively large quantity of diffusible coloring matter, stain the reactive exudate, which is creamy in consistence, a bluish green color. Pus arising in the course of pyocyaneous infections is of a very characteristic musty unpleasant odor. It is usually possible to diagnose infection of wounds by this bacterium without visual examination.

Organisms such as those belonging to the "colon" group and many of the anaerobes, which liberate foul-smelling gases in the process of growth, are often suggested by the presence

of the odor of the pus alone. Since proteolytic microorganisms of the aerogenes and malignant edema groups actually destroy and liquefy the tissues in which they are growing, the discharges from such tissues are usually watery and blood-stained.

Chronic Inflammation

As stated in the last section, if the irritant be sufficiently toxic, there is induced in the tissues a reaction which is essentially exudative in character. The participating cells, as well as the serum, are derived from the circulating blood. Such a type of inflammation takes place only in the presence of certain virulent bacteria and a comparatively small number of toxic inanimate substances. On the other hand, substances which are mildly irritating to the tissue cells do not determine the accumulation, to any considerable degree, of polymorphonuclear leucocytes. As a rule, in such cases, very large accumulations of extravascular serum do not take place. The presence of mildly irritating substances does, however, induce a reaction which is characterized by the collection at the site of the foreign material of cells of the lymphoid and macrophage groups and an increase in the flow of fluid through the part; although, only infrequently does it result in the development of a "pitting" edema.

Since the cells of these groups increase at the inflammatory focus chiefly, if not exclusively, by multiplication *in situ*, reactions of this type are known as proliferative lesions.

In order to study the phenomena which characterize this type of reaction, let us note the changes which occur in the presence of a collection of dead tissue cells, such as occur following the cutting off of blood supply (infarction). Since the necrotic tissue cells occupy space which nature attempts to replace by more or less useful tissue, and since also the dead cells themselves assume irritant properties on account of the development of protein degradation products, it is necessary that they be removed. The means adopted for the absorption of such material is the proliferative reaction, and absorption is

accomplished by the accumulation of lymphoid and plasma cells and macrophages, including, if the occasion demand, the so-called foreign body giant cells.

There is some doubt as to the origin of these cells. There is, however, no doubt but that they increase in number as the result of proliferation, *in situ.* As a class, these types of cells are phagocytic for tissue debris and produce enzymes which lead to liquefaction of the digestible portions of necrotic material. This broken down or dissolved material is removed from the part, as a result of the increased flow of fluid through the part. This increase in blood circulation is supplied by means of an increase in the number of blood vessels by budding. In consequence of the accelerated blood flow through the part and an increase in the amount of fluid poured into the tissues, there occurs an active efferent lymphatic flow which carries with it soluble products of cell disintegration and also a larger or smaller number of phagocytic cells containing insoluble or poorly soluble substances. These ingested materials, whether bacterial or of other origin, are commonly carried to, and deposited in, the neighboring lymphatic glands.

Other macrophages, having phagocytized as much foreign material as they can contain, wander into the surrounding tissues where, if insoluble and nondigestible, the substance remains, upon the death of the cell, as a deposit.

Coincident with the absorption of portions of the necrotic mass there commences fibroblastic proliferation of the fixed tissue cells; in other words, reparative changes progress hand in hand with the inflammatory reaction. Occasionally, also, there is an attempt made on the part of the tissues to replace the destroyed tissue by parenchymatous elements. It is but rarely that this effort at regeneration is successful in man.

The microscopic lesion in chronic inflammation consists of an increased number of blood vessels, moderate interstitial edema, and masses of proliferated lymphoid and plasma cells, and larger or smaller numbers of macrophages. Surrounding

the inflammatory focus there is usually a fibrous tissue zone, and, since a certain quantity of the material removed by the lymphatics retains its toxicity, there is frequently exhibited a perivascular infiltration at a considerable distance from the original focus of irritation and a series of changes in the neighboring lymph nodes.

This type of reaction is developed in order that useless inert tissue, such as necrotic cells, hemorrhagic material, and sterile exudates, may be absorbed. A similar reaction is induced by the presence in the tissues of microorganisms whose virulence has been attenuated, or by those whose essential or allergic toxicity is relatively low.

As a means of combating bacterial or protozoan infection, the proliferative type of lesion is relatively ineffective. We thus find that, in the presence of bacteria which induce no polymorphonuclear exudation, the pathogenic process runs a very chronic course. It is the surgeon's duty to attempt to stimulate, if possible, the development of exudative lesions, if such can be accomplished without harmful sequelae. It would appear that it is only insofar as polymorphonuclear phagocytosis can be stimulated, that the eradication of focal bacterial collections can be accomplished by the tissues.

The usefulness of bacterial vaccine injections in chronic diseases depends upon the fact that acute inflammatory reactions may be thereby induced in the foci harboring the bacterioproteins. Clinically, the chronic, or proliferative, lesion is differentiated from the acute reaction by the relative absence of redness and "pitting edema," and by its greater density or firmness upon palpation. The characteristic hard sore or chancre of syphilis is typical of a pure chronic lesion; its firm consistence is due to enormous infiltration of the tissues with lymphoid and plasma cells, and the absence of necrosis and free serum collections. Since, moreover, reparative changes are more likely to occur along with chronic reactions, the lesion attains density through the formation and contraction of fibrous tissue fibrils.

In certain subacute conditions, especially those stimulated

by the presence of the gonococcus and bacilli of the colon groups, polymorphonuclear leucocytes as well as eosinophiles infiltrate the tissue in comparatively large numbers.

Granulomata

Certain specific irritants, more particularly the B. tuberculosis, B. leprae, and the Treponema pallidum, and certain of the parasitic yeasts, induce reactions of a characteristic type. Inasmuch as, in a superficial way, the reactive tissue resulting from the activity of these microorganisms resembles granulation tissue, and as there is a tendency for a definite mass of permanent new tissue to persist at the site of the reaction, the lesions resulting from the presence in the tissues of these organisms are called granulomata. Although all the granulomata have many features in common with one another, and with other types of chronic inflammation, there are important differences between them which can be made use of in histologic diagnosis, and which help to explain the clinical course of the affections in which they are found.

Tuberculosis

Following invasion of the tissue by the tubercle bacillus, there occurs a reaction which is essentially proliferative in type, and which is accompanied by lymphoid and plasma cell infiltration. These cells surround the foci of bacilli, and increase in number, forming a small nodule, in which the cells are arranged in a concentric manner. At the same time a different type of cell appears—a mononuclear cell with a pale staining vesicular nucleus and moderately abundant acidophilic cytoplasm—known as an "epithelioid" cell, since it resembles certain types of epithelium. This cell is found, characteristically, in the center of the lymphoid and plasma cell masses. As the result of two factors, namely, the separation of the central cells of the proliferated mass from the nutrient blood vessels and the presence of the microorganisms, necrosis occurs in the inner zones. The necrotic mass, known as caseous material, is composed, therefore, of dead fixed tissue and in-

flammatory cells together with a larger or smaller amount of serum.

The characteristic tubercle consists of a central area of necrosis surrounded in turn by epithelioid cells and by lymphoid and plasma cells. Only outside the latter zone are blood vessels present. As the result of the fusion or coalescence of tubercles, large irregular areas of necrosis may result.

If the cellular reaction is successful in controlling the activity of the bacilli, an effort at repair ensues. Fibroblasts arise in the surrounding fibrous tissue, with the result that the tubercle may be completely walled off, or even replaced by fibrous tissue similar to that which results from the granulation repair of tissue destroyed by other agents. Under such circumstances calcium salts are often laid down in the dead tubercle, so that larger or smaller calcareous masses result.

It must be remembered, also, that, if the individual be sufficiently sensitive to the tuberculo-protein, polymorphonuclear leucocyte invasion may take place and abscesses may develop.

Although the amount of serum which accumulates in the reaction against the tubercle bacillus is usually minimal, this is by no means always the case. An increased collection of fluid is determined apparently by two factors, namely, the virulence of the bacillus (see page 46) and the ease with which fluid may accumulate without increasing to any considerable degree the extravascular tension. Thus, in the pleural and peritoneal cavities, as well as beneath the arachnoid membrane, very large collections of serum not infrequently develop.

Syphilis

The lesions which result from the presence of the Treponema pallidum in the body are of several types. The commonest type, which is characteristic of the primary and the majority of the secondary manifestations, is a simple lymphoid and plasma cell proliferative lesion, accompanied by dilatation of vessels and a moderate amount of serum exudate. In general, the plasma cells form the greater proportion of the new tissue.

The spirochete appears to have a tendency to affect blood vessels in a characteristic manner. The perivascular tissues are especially attacked, and are the site of an intense plasma and lymphoid cell infiltration. In addition, the endothelium of the smaller arterioles and the capillaries is stimulated to proliferate. As the result of one or other or of both of these reactions, the lumen of the smaller vessels is frequently materially encroached upon and sometimes obliterated. Following such vascular occlusion the tissues supplied are deprived of nourishment and necrosis takes place. This is the type of lesions which leads to atheroma of the aorta and aneurysm formation. In these affections the vassae vasorum are primarily affected.

The vascular changes take place in all stages of the disease. In the earlier and more active lesions necrosis rarely occurs, since there is commonly sufficient increase in the number of vessels to protect the tissues from complete ischemia. In the later stages, those customarily termed tertiary lesions, comparatively large infarctions of tissue not infrequently arise in this manner, as for instance in cerebral thrombosis. Many of the lesions commonly termed gummata are of this nature.

There is a third syphilitic lesion for which this special designation (gumma) should, in my opinion, be reserved. This lesion is similar in appearance to that of tuberculosis and is the true gumma of syphilis. There is the same central area of necrosis with epithelioid and small round-celled layers.

As with other chronic inflammatory lesions, following the destruction of the irritant by mercury, salvarsan, or by partial eradication by the tissue cells, reparative fibrotic changes take place. It is the chronic reaction, followed by fibrous tissue repair, which accounts for many of the late manifestations of syphilitic infection, e.g., tabes, interstitial nephritis, and cirrhosis hepatis.

Leprosy

The histologic lesion induced in the human tissues by the presence of the B. leprae consists of collections of lymphoid

and plasma cells, followed by proliferation *in situ* of large cells resembling in appearance endothelia. These cells have a pale-staining, nongranular protoplasm, and a more or less oval or kidney-shaped nucleus with a distinct chromatin network. The protoplasm of most of these cells is vacuolated: in some the vacuoles are single and large, completely filling the cell and pushing the nucleus to one side; in others the vacuoles are multiple and small. In thick sections the vacuoles from one cell can apparently be traced by direct continuity to another. These vacuoles represent a degenerated area within the cell cytoplasm. They are the result of the action of the bacilli which are usually found within the clear area.

In the earlier lesions, and especially in those experimentally produced in mice, the plasma and lymphoid cell elements are the predominant cells present. I am of the opinion, as already stated,[1] that lymphoid, plasma, and "epithelioid" cells are intimately related to one another; the last mentioned is developed in larger numbers in proportion to the duration and intensity of the irritant.

Mast cells are usually present as well as eosinophiles. Neutrophile-polymorphonuclear leucocytes are not infrequently found in small numbers, and in one case, reported in 1911,[2] they were present in such numbers as to form distinct abscesses, although no organism other than the leprosy bacillus was present in the lesion (see Immunity).

There are also usually present, especially in the early lesions, giant cells of the Langerhans type, which exhibit a large amount of irregular granular protoplasm and contain numerous nuclei. These are usually situated towards one end of the cell and are frequently arranged in a horseshoe manner. Division by amitosis in these cells is frequently seen. In cells of the epithelioid type karyokinetic figures are occasionally found. In addition to these distinct giant cells, irregular agglomerations of cells of the epithelioid type occur. The difference in the arrangements of the nuclei in such cells and the

[1]Gurd: Jour. Med. Research, 1910, xxiii, 151. Jour. Path. and Bacteriol., 1911, xvi, 1.
[2]Gurd: Jour. Inf. Dis., 1911, viii, 39.

absence of granular protoplasm make their differentiation from the true giant cells easy.

A feature differentiating the giant cell of the leprous lesion from that found in tuberculosis is the presence in the great majority of cells of round vacuole-like spaces. These spaces vary in shape from spherical to sausage-shaped bodies, and vary in size from 4 to 100 or more microns in thickness. Many vacuoles are present which do not show any nucleolated cell body, although in all cases there is a narrow margin of granular protoplasm. This appearance is the result of the cutting of the cell in such a way that the nuclei are not brought into the section. That this is the case can be proved by means of serial sections. A certain number of cells of this nature appear to have been completely replaced by the vacuole-like body.

Practically all the bacilli seen in sections of leprous tissue are situated within the protoplasm of the tissue cells. Not only are they present in the two types just described, but they are also found lying within lymphoid, plasma, and connective tissue cells, in the superficial layers of the corium, and in the fat cells. They are also constantly present in the cells of the adventitia of the vessels. In many instances the masses of bacilli are continuous from one cell to another, forming in this way irregular branching bodies.

A narrow zone of normal-looking corium is always present immediately beneath the epidermis. Although this zone is free from inflammatory cells, bacilli are not infrequently found.

Material scraped from the surface of an incised leprous tubercle always contains enormous numbers of leprosy bacilli. A certain number of scattered organisms are usually present, though the greater number are in the form of spherical masses (globi) and irregular branched collections. As a rule no cell bodies or nuclei are noted in direct relationship to the masses of bacilli. The shape of the cell body, however, and the spaces occupied by the nucleus of the cells from which the branched forms are derived can always be made out. That only a small number of nuclei and cell bodies is found is explained readily by the study of the tissues themselves. Everywhere between the epithelioid cells are found collagenous fibrils, which act as

a support to the cell protoplasm. The epithelioid cells, and more particularly the giant cells, have an extremely irregular outline, numerous prolongations of the protoplasm protruding everywhere between neighboring cells. In view of this fact and the fact that the cell membrane, as demonstrated in stained preparations, is very thin and the vacuoles filled with bacilli are proportionately large, it is easy to understand why the bacilli are thrown off from the cells as a result of the trauma of incision; whereas the cells themselves, owing to their projections, are more firmly attached and do not exude so readily.

Other Granulomata

In addition to tuberculosis, leprosy, and syphilis, there are a certain number of diseases in which a lesion of the granulomatous type is found. Infection by the actinomyces, sporotrichoses, B. mallei, and blastomyces, may produce lesions which closely resemble those which characterize tuberculosis. Fortunately these infective agents are comparatively readily demonstrated and recognized, so that they do not as a rule offer difficulties in diagnosis.

Protective Effect of Macrophages upon Certain Microorganisms

Although hypersensitiveness to bacterioprotein usually determines protection of the tissues from infection by the bacterium, it occasionally happens that, in consequence of certain physicochemical characteristics of the bacterial cell body, such is not the case. I[3] have already expressed the opinion that with regard to infection of certain animals, notably goats, by the leprosy bacillus, the reaction which develops in consequence of a moderate degree of hypersensitiveness to the bacterial protein may suffice to permit the bacterium, under conditions of massive experimental inoculation, to obtain a foothold.

Attempts to infect normal goats with the leprosy bacillus (Duval) were attended uniformly by failure upon the first

[3]Duval and Gurd: Jour. Exper. Med., 1911, xiv, 181.

attempt. Nor did inflammatory reactions of any sort occur in the tissues, if moderate doses were employed. If, however, the animals had been rendered hypersensitive by means of the introduction of one or more massive doses of bacterial emulsions or extracts, subsequent subcutaneous injections of moderate doses of viable bacilli were followed by the appearance of inflammatory nodules. These nodules were characterized by hyperemia and mononuclear cell accumulations, as well as by the presence of moderate numbers of multinucleated cells. These last mentioned cells appear to ingest the bacilli and, having ingested them, are unable to complete their destruction. The bacilli, therefore, continue to live, and apparently to multiply, in the protoplasm of the giant cells, and form the characteristic globi.

My explanation of these observations is that the leprosy bacillus (Duval) is unable to live in the tissues if the goat is exposed to the circulating body fluids, nor is it irritating to the normal goat tissue. In consequence there is an absence of inflammatory reaction about the foci of bacilli. If, on the other hand, the animal has been rendered moderately hypersensitive, the characteristic macrophages accumulate.

These cells ingest the bacilli, but are unable to bring about their devitalization. The ingested bacteria, therefore, continue to proliferate in the protoplasm of the cell. At the same time the microorganisms are protected from adequate action of the substances in the serum, which, experiments appear to prove, are injurious to their metabolism. The bacilli consequently are enabled to persist and to grow in the cytoplasm of the giant cell. The typical globi which characterize the tissues are thus produced.

The extent to which other microorganisms are thus protected by inclusion in the cytoplasm of cells which are not capable of destroying the invading virus is not known. The microscopic appearance of lesions consequent upon infection by the blastomyces, in which budding cells are more frequently seen in sections of macrophages, suggests that this may be the case in infection by this yeast.

CHAPTER XXIV

ANAPHYLAXIS IN MAN

Reactions to the Parenteral Introduction of Horse Serum in Man—Serum Sickness

In man the parenteral introduction of foreign protein[1] in the form of antiserum is accompanied in a large proportion of cases by local and constitutional manifestations of irritation or intoxication. Such symptoms consist, in the majority of cases, in the development of urticarial and erythematous eruptions, joint or muscle pains, pyrexia and occasionally vomiting.

Such symptoms, though they may be annoying to the affected individual, and although, occasionally, the onset of an unexplained pyrexia in the course of the treatment of the wounded may cause anxiety to the surgeon in charge, are of comparatively little importance. Unfortunately, however, there occasionally occur, within a short time after the injection of the serum, more serious reactions. The symptoms in these cases comprise collapse, tachycardia, drop in blood pressure, unconsciousness, and occasionally difficulty or arrest of respiration with consequent cyanosis and air hunger. Such cases are often fatal.

It is evident from a study of human cases that three types of reaction are exhibited, namely, urticaria and cellulitis, as in the rabbit; splanchnic dilatation, as in the dog; and respiratory anaphylaxis, as in the guinea pig. It would appear that, both as a primary cause of death and also as subjecting the patient to the danger of a delayed reaction, the splanchnic type is of most importance.

When an injection of horse serum is made into the human

[1]For a more complete discussion of this subject, the reader is referred to an article by the author, Gurd: Arch. of Surg., 1921, ii, 409.

tissues, various results may be noted depending upon the state of the tissues in relation to the serum protein.

1. If the individual be normal, no immediate result is noted, but his tissues are stimulated to elaborate a substance (first order antibody) which has the property of altering the protein molecule so that it acts as a tissue irritant.

During that period immediately following the introduction of the horse serum the amount of available antibody is not sufficient to liberate, at any one time, a sufficient amount of toxic substance to irritate the tissues either locally or constitutionally. After the sixth day, the amount of antibody available has reached such concentration that a larger quantity of protein is split. In consequence, the focus into which the serum has been injected becomes irritating. As the result of irritation hyperemia develops so that a large amount of blood is brought into contact with the serum collection. As a result, then, of a greater concentration of first order antibody, in the body fluids, and an increased circulation through the focus, there is a marked increase in the amount of irritant substance developed. Symptoms of constitutional intoxication then occur. These manifestations consist of fever, skin eruptions, swelling of the synovial membrane with consequent joint pains, and occasionally nausea or vomiting, and diarrhea.

At the site of serum injection and in the neighboring lymph nodes, evidences of inflammation, hyperemia, interstitial edema, pain and tenderness are noted.

2. If the individual is hypersensitive to horse serum, either because he has previously received parenteral injections of horse serum, or from any other cause, the injection of the antigen into his tissues is immediately followed by a reaction between the first order antibody already present and the injected protein.

The severity of the reaction which takes place depends upon several factors, viz.:

a. The degree of sensitiveness of the patient.

b. The route of injection (intradermic, subcutaneous, intramuscular, intrathecal or intravenous); this is important, since

depending upon the nature of the tissue into which the serum is injected, there will be a variation in the available proportion of the amount of antibody present in the body.

c. The amount of serum injected.

d. The rate of injection. If the serum be very slowly injected into the blood stream, the total quantity of antibody available may be exhausted by a very small amount of serum. The amount of serum which is capable of absorbing all the available antibody may not be able to supply a sufficient quantity of toxic product to materially injure the individual.

2-a. If the individual be very sensitive, the route of injection be intravenous, and the dose injected sufficient to produce an excessive amount of anaphylactic irritant, there occurs an almost instantaneous liberation of the irritant product with immediate manifestations of grave intoxication of the individual. Death may supervene within a comparatively few minutes, either as the result of drop in blood pressure and arrest of circulation, as is seen in anaphylactic shock in the dog, or the reaction which occurs in respiratory, as in the guinea pig. In man it would appear that this respiratory type of reaction, though very terrifying, is less likely to lead to a fatal outcome than is the splanchnic reaction.

In a certain proportion of cases which are shocked by the parenteral introduction of serum there occurs the recurrent splanchnic reaction, after the lapse of several hours, which may prove fatal.

2-b. If the serum be injected intradermally in small quantities, e.g.,0.25 c.c., the reaction which occurs is almost entirely focal. It commences within two or three minutes and is characterized by a well marked cellulitis of the tissues surrounding the injected area; it is accompanied, not infrequently, with lymphangitis and lymphadenitis.

2-c. If the serum be injected intramuscularly, it is brought in contact with less blood than if given intravenously; in consequence the reaction which occurs is, other things being equal, less severe and more prolonged than following intra-

venous administration, though more severe than following intradermic injections.

3. If the individual has been desensitized by a recent injection of horse serum (a few hours to four days previously), the injection of a subsequent dose finds the tissues free from any considerable amount of first order antibody. In this event, the injected serum is not immediately altered, as in the hypersensitive individual, but remains unaltered pending the accumulation, through the activity of the tissues, of a fresh supply of antibody. Since the tissues have already been stimulated to produce the antibody, the development of amounts sufficient to cause the liberation of an amount of irritant capable of injuring the tissues, requires a shorter period than in the normal individual. In consequence, the outbreak of clinical manifestations of irritation is not delayed for eight or nine days, but appears at the expiration of a shorter period.

4. If the individual has received frequent large doses of serum within a few weeks or months, he is to a certain extent (tolerant) immune to the toxic effects of the reaction between the first order antibody and the serum protein. Such a case may be injected by any route with a small dose of serum without the development, either immediately or later, of manifestations of intoxication. In such a case the injected serum protein is acted upon (split) by the first order antibody, but there is also present in the tissues a substance which is capable of neutralizing the toxic product—second order antibody.

The fact that it is possible to inject hypersensitive individuals with a small dose of the protein to which they are hypersensitive, without the development of symptoms of tissue irritation, must be borne in mind. Should the injection of a test dose in an individual, who is suspected to be hypersensitive to horse serum, e.g., an individual known to be asthmatic, or to have previously received injections of horse serum, prove negative, the evidence thereby gained should not be accepted as final. The person should again be injected with a second test dose, larger in quantity, before it is assumed that he is not hypersensitive.

In my opinion, the fact that the hypersensitive individual may also be tolerant to the irritant product of the first order antibody antigen reaction, and consequently his hypersensitiveness may not be evident if too small an amount of the test serum is introduced, is an important argument against the exclusive employment of the cutaneous method of testing for hypersensitiveness as used by Walker and others. By the employment of the subepidermal method of injection, more clean cut reactions are induced, and the quantitative element which is lacking in the cutaneous method is obtained. The method, moreover, is of real value in commencing desensitization of the individual.

Novotny and Schick[2] report the successful transference of passive anaphylaxis to guinea pigs with the serum of two children who had received injections of horse serum, seventeen and twenty-three days, respectively, previous to being bled; these, however, are the only positive results in a considerable number of experiments. They were unsuccessful in attempts to transfer anaphylaxis passively from dog to rabbit, and from guinea pig to rabbit.

Determination of Hypersensitiveness and Method of Desensitization.—At the present time, there are not sufficient data available to permit one to state with positiveness the optimum dosage of horse serum which should be employed in the determination of hypersensitiveness or in the induction of desensitization. I have employed a method which has apparently proved satisfactory. For the test dose, 0.25[3] c.c. of serum is introduced subepidermally. In performing the test, I have been accustomed to employ a very fine needle which is introduced through the skin and made to penetrate the skin again at a distance of a centimeter or more from the original puncture. As soon as the point of the needle is visible through the epidermis, the serum is injected. In this way, a small white wheal is formed, which is the center of the subsequent reaction.

[2]Novotny and Schick: Quoted from Anderson and Frost, Trans. Cong. Am. Phys. and Surg., 1910, viii, 430.

[3]In one case 0.25 c.c. of horse serum introduced subepidermally was followed by the exhibition of somewhat alarming symptoms.

It is of the utmost importance that the fluid should not enter a vein. Such an accident can be guarded against by attempting to withdraw the plunger of the syringe before injecting the fluid.

In sensitive individuals, the reaction commences almost immediately (from 20 to 180 seconds), and consists in its first stage of an enlargement of the original wheal, usually in a radiating manner. Not infrequently at this stage, the point of injection becomes itchy. Surrounding the definitely raised area, there appears within from three to five minutes a hyperemia zone, 1 to 5 cm. in diameter, which rapidly increases in size until it attains its maximum size about one-half hour after injection.

The results of experiments in one case that I studied proved that in certain hypersensitive individuals, 0.25 and even 0.5 c.c. is too small a dose to prove hypersensitiveness in individuals who have recently received large doses of serum and who are, in consequence, tolerant.

Should there be no reason for suspecting the patient to be highly sensitive or perhaps tolerant, a negative reaction to this amount (0.25 c.c.) may be accepted as proof that the individual is not hypersensitive to an important degree. On the other hand, should the fact that the patient had recently received one or more doses of serum lead one to suspect that he might be both hypersensitive and tolerant, the injection should be repeated about one hour after the first, and a larger quantity, 1 to 1.5 c.c. of serum should be employed.

Desensitization of animals sensitized to milk proteins has been accomplished by the rectal administration of milk. (Besredka.) This is a method which may perhaps be of occasional value in desensitizing patients to whom it is necessary to give doses of a therapeutic serum and who are suspected or known to be hypersensitive to horse serum. The only objection to the method is that many hours are necessary for desensitization to take place.

Friedberger and others have shown that desensitization without the manifestation of anaphylactic shock may be induced

through the slow administration intravenously of very dilute antigen. For this purpose, an hour or more must be consumed in the administration of the dose required to desensitize.

Treatment of Anaphylactic Reaction in Man.—In the treatment of the mild forms of the reaction, little is necessary. In my experience, the discomfort arising from the urticaria is usually relieved by the administration, either before or after its development, of calcium lactate or chloride, followed by a dose of magnesia. I have been accustomed to give the patient a mixture containing 1 dram of calcium chloride, one-fourth of the mixture to be taken every fifteen minutes; and at the end of the hour a dose of 4 drams of milk of magnesia is given.

In the treatment of the severe cases of splanchnic dilatation, posture, including compression of the abdomen, should be employed. Fluids, probably best in the form of hypertonic glucose (10 per cent) solutions or gum acacia, are administered intravenously. Epinephrin, in doses of 5 or 10 minims (1:1000) intravenously, should be employed, if available, for its splanchnic effect. In the event of recurrence of symptoms, further injections of epinephrin must be employed.

Although probably less effective than suprarenal extract, pituitary extract is, I believe, of value. It is probable that although its effect is less prompt than epinephrin, the best results are obtained by employing the latter preparation intravenously and injecting the pituitary extract in doses of 1 c.c., subcutaneously.

In cases of respiratory reaction, epinephrin should also be employed and, in addition, oxygen should be administered. As suggested by Auer and Lewis, the effect of atropin upon the smooth musculature is of real value in relieving the dyspnea. This drug should be administered in doses of $\frac{1}{100}$ grain. In view of the fact that animals are apparently more resistant to fatal shock when under the influence of anesthesia, and in view of the excellent results obtained by Munro (see p. 96) in a case reported by him, chloroform or ether may

well be administered. Artificial respiration should also be employed.

Clinical Examples of Hypersensitiveness to Nonbacterial Proteins

As early as 1911 von Pirquet[4] discussed, as manifestations of the allergic phenomenon, satinwood dermatitis, egg albumin hypersensitiveness, buckwheat poisoning, insect poisoning, and eclampsia, as well as hay fever and serum sickness.

Hay fever is an example of a local allergic reaction in individuals sensitive to pollen protein. The same is true of the asthmatic attacks from which certain individuals suffer when in contact with horses or cats or following the ingestion of special foods.

Coca objects to the inclusion of hay fever as an expression of hypersensitiveness to plant pollens on account of the negative results which have been obtained in efforts to passively sensitize guinea pigs. As pointed out by Wells, this is of little significance if we consider that even the serum of guinea pigs, highly sensitized to a foreign protein, often contains too few free antibodies to confer passive sensitization upon other guinea pigs. The tissues of a man may be highly sensitized to a foreign protein without there being free antibodies in his blood to produce passive sensitization.

It has been previously pointed out that under certain circumstances the individual may become sensitized by means of the alimentary, or respiratory, tract. It is believed that so-called "food idiosyncrasies," such as that evidenced by certain individuals towards articles of food such as strawberries, eggs, cow's milk, cheese, fish, pork and buckwheat, is due to some change in the physiology of the alimentary tract whereby these materials are not properly dissociated before their absorption into the system.

Ramirez[5] has reported a case of a man who had never had asthma, hay fever, urticaria, or any similar condition indicat-

[4]von Pirquet: Arch. Int. Med., 1911, vii, 284.
[5]Ramirez: Jour. Amer. Med. Assn., 1919, lxxiii, 984.

ing hypersensitiveness to proteins, who received 600 c.c. of blood as a transfusion from a man who had typical horse asthma. The donor gave a cutaneous reaction to horse dandruff in 1-50,000 dilution. Two weeks after the transfusion the recipient went for a carriage ride. Within five minutes he had a typical attack of asthma. A skin test gave a positive reaction to horse dandruff diluted 1-20,000 but not to numerous other proteins.

Hypersensitiveness to Foodstuffs.—Talbot[6] has investigated extensively the importance of the intestinal tract in sensitization to foodstuffs. In his opinion (as well as that of others), asthma, eczema, and certain explosive forms of gastrointestinal disturbances are often due to hypersensitiveness to articles of diet. In children and young adults these diseases are not uncommon. As a rule, as the patient grows older, tolerance to the protein is developed and clinical symptoms cease.

Lust and Hahn[7] have shown that in a small percentage of babies with digestive disturbances precipitin to the casein of cow's milk is demonstrable in the serum. Schloss and Worthen found that although the normal intestinal tract ordinarily does not permit the passage of undigested foreign protein, certain forms of gastrointestinal disorders may permit of the absorption of protein in an undigested, or partially undigested, state. Foreign proteins may under these conditions appear in the urine.

The difficulty of determining the cause of the exciting agent in these conditions is due to the fact that, not infrequently, they are caused by bacterial antigens and not by native foodstuffs.

Ascoli, Oppenheimer, and others have observed that if animals be fed individual proteins in large quantities, the same protein may be subsequently discovered, not only in the blood, but also in the urine. As a means of identification they employed the precipitin reaction. This type of experiment appears to indicate the method of active sensitization of individ-

[6]Talbot: Boston Med. and Surg. Jour., 1918, clxxix, p. 1.
[7]Hahn: Jahrb. f. Kinderh., 1913, lxxvii, 405.

uals to foodstuffs. In view of the extremely minute doses of antigen necessary to sensitize when parenterally introduced, it is quite conceivable that, from the intestinal content, a sufficient amount of foodstuff protein may be absorbed to actively sensitize the individual.

Wells has been successful in inducing the hypersensitive state in guinea pigs by feeding specific proteins to young animals. It will be noted from the following experiments, which are quoted from Wells, that not only may hypersensitiveness be thus developed but that, in certain instances, tolerance is also induced. In the routine testing of individuals by means of the allergic reaction to common articles of diet, it is frequently noted that patients react to proteins which are habitually included in their dietary without untoward effects. Such an observation may be explained by the assumption that no unaltered protein is, in fact, absorbed into the tissues, but is more likely to be dependent upon a condition of tolerance having been induced.

In Wells' experiments, "guinea pigs bred from mothers fed with oats, were, as soon as weaned, put upon a diet of egg albumen and carrots. Other young pigs from the same stock were raised upon oats and carrots. The latter animals after reaching a weight of 250 to 300 grams, did not give anaphylactic reactions when injected with 0.05 gram of a protein obtained from raw oats, and if given small doses, such as ordinarily given for sensitizing, they were not rendered sensitive to subsequent injections of 0.05 gram of oat protein.

"Some of the pigs which were raised to 200 to 250 grams weight without oats were found to give a typical reaction of moderate severity when injected once with 0.05 gram oat protein. These reactions apparently resulted from passive sensitization conferred by the mother. Others gave no reaction. After the animals fed without oats were somewhat older, 350 to 400 grams, they reacted much less strongly, or not at all, to oat protein, as if this inherited passive sensitization were passing off, as passive sensitization normally does; such pigs, if given sensitizing doses of oat protein were found to be

sensitive to this protein three weeks later and gave well-defined reactions of moderate severity.''

Hence, says Wells, the conclusion seems warranted that if guinea pigs are raised on oat proteins they cannot be made to give anaphylactic reactions with oat proteins, but if raised without oats, they may be sensitized to oat protein, just as they can be to other proteins not usually in their food. These experiments support experience obtained previously with zein, that guinea pigs become immune (tolerant) to the chief vegetable proteins of their food.

In another experiment Wells found that guinea pigs raised from the time of weaning on a diet of egg protein and carrots were found to give strong anaphylactic reactions when injected with egg albumen between the thirtieth and sixtieth days, but later they reacted less strongly, and after the one hundredth day of feeding they gave but slight reactions to 0.1 gram dried egg albumen. At this time sensitization with egg albumen can be obtained to only a slight degree. When such guinea pigs were given injections of egg albumen they showed but slight reaction to a subsequent dose of egg albumen, while control pigs fed on oats and carrots gave severe, usually fatal, reactions to corresponding injections of egg albumen. Apparently, the daily absorption of animal protein in the food at first renders guinea pigs hypersensitive to this protein, but if the feeding is kept up for a long enough period the animals become refractory (tolerant) to the food protein and are so immunized that they cannot be sensitized to this protein. (Wells.)

''A series of guinea pigs, which were raised on bread and cow's milk for two weeks, were found at the end of this time to be still highly sensitive to milk. They died promptly when given 1 to 3 c.c. of milk intraperitoneally. Apparently this length of feeding is not sufficient to render guinea pigs immune to milk.''

Greer[8] believes that there is strong evidence that in infants suffering from certain types of gastrointestinal disorders, there

[8]Greer: Arch. f. Pediat., 1917, lxxxiv, 810.

is increased permeability of the intestinal wall to incompletely digested lactalbumin and to a less extent of caseinogen. Sensitization consequently takes place. This author's conclusions were based upon experiments in which infants were subjected to intradermic injections of cow's milk.

Hypersensitiveness to Serum.—Coca frankly eliminates serum sickness entirely from the category of anaphylactic reactions. He does not consider that any of the conditions characterized by cutaneous hypersensitivity such as the tuberculin reaction, pollen reaction, food and drug idiosyncrasies, fall into the domain of anaphylaxis. Kolmer on the other hand believes that the cutaneous reactions, such as those which are exhibited following the introduction of tuberculin, luetin, horse serum, etc., into suitable individuals are true anaphylactic skin reactions and that they are due to the interaction of a specific anaphylactic antibody and specific anaphylactogen with the formation of diffusible irritants capable of producing acute hyperemia, edema and leucocytic infiltration of the skin. He thus accepts the theory of local anaphylaxis (allergy) as consequent upon the formation of an irritant by tissue reactions to foreign proteins introduced into the skin.

That serum disease is related to anaphylaxis is given definite support by the observations of C. W. Wells. This author found that in several persons who developed such manifestations the precipitin titer fell to rise again when the rash faded, as if the precipitin had been found in the skin and thus caused a local anaphylactic reaction. Also, Weil observed in human serum sickness a fall in blood pressure and a decrease in the coagulability of the blood, thus adding to the resemblance of this condition to true anaphylactic reactions (Wells).

Krause has noted that bovine serum does not cause serum sickness with as great frequency as does horse serum. Wells makes the suggestion that the explanation may be that man, since he uses beef protein as a main article of diet, may develop an actual immunity (tolerance). He has found that

guinea pigs do become immune to the proteins which form the chief elements of their diet.

In respect of certain acute serum reactions Wells, referring to death of a person suffering from horse asthma a few moments after receiving the injection of horse serum, states: "It seems evading the obvious to attempt to interpret the occurrence in any other way than as true anaphylactic shock, resulting from a specific antigen-antibody reaction; and as such a sensitized person usually exhibits also typical acute local reactions immediately after intracutaneous introduction of the most minute amounts of horse serum, or of other horse proteins as well, it seems difficult to deny, at least in such a case, that the cutaneous reaction of hypersensitiveness represents a true specific antigen-antibody reaction, and is a true manifestation of anaphylaxis. Furthermore, it is sometimes possible to desensitize a person to the protein to which he is sensitive, removing both the systemic and cutaneous reactivity."

There are still many phenomena of intoxication which have not yet been subjected to sufficiently careful investigation to make it possible to make positive statements regarding their nature. Among the most important are the irritant effects occasionally exhibited by transfused blood, and by the injection of numerous protein materials which are employed under the heading of "nonspecific protein therapy." The irritative effects noted in these conditions may be due to either protein split product intoxication or to embolic occlusion of vessels. The emboli responsible for the vessel occlusion may be developed as the result of agglutination of red blood cells, fibrin formation or flocculation of colloids.

Loeb, Strickler, and Tuttle,[9] as the result of an investigation into the cause of death which follows injection of normal dog or beef serum into rabbits, have published the following conclusions: "Death following the injection of foreign serum is brought about by obstruction of the pulmonary circulation

[9]Loeb, Strickler and Tuttle: Quoted from Zinsser: Infection and Resistance.

either by heaps of agglutinated erythrocytes or by fibrinous plugs. Dog serum and beef serum represent two different types. In the case of dog serum hemolysis of the blood cells of the recipient liberates substances attached to the stromata, which hastens coagulation. In consequence fibrin is formed which is carried into the pulmonary vessels and occludes them. In the case of beef serum death is due to hemagglutination.''

The so-called traumatic fever, which so frequently accompanies injuries, and surgical operations, is apparently the result of the absorption of toxic autolytic products of hemorrhagic or serous exudates. Although such intoxications are obviously not examples of anaphylaxis, there is much which suggests that the irritant product, responsible for the clinical symptoms, is closely related to that which is explosively developed in anaphylaxis.

Recent experiments by Carrell regarding the absence of reparative changes in wound healing, if the tissues are protected from any form of irritant, indicate that the irritative property assumed by extravasated blood during autolysis serves a positively useful purpose in stimulating cellular activity.

Kohler, Moldovan, Doerr, and others found that if some means is taken whereby clotting of blood is delayed or prevented, as for instance, receiving it into paraffined vessels, by defibrination, or by citrating, it may become toxic automatically, and cause death upon reinjection into animals of the same species, or even into the same animal from which it was taken. DeKruif extended observations which had been made by Slatineau, and Ciuca, and showed that rabbit blood can be rendered toxic and even fatal to guinea pigs and white rats, if transfused in the preclot period, or after defibrination. Guinea pig blood transferred unchanged within three minutes, or defibrinated, or occasionally even the serum of such blood obtained by rapid clotting and centrifugation, was often fatal to animals of the same species.

Zinsser summarizes DeKruif's conclusions regarding this phenomenon, as follows: ''The spontaneous toxicity of nor-

mal blood develops in a similar manner as does the toxicity of blood treated with agar, peptone, etc. Poison production and fibrin formation go hand in hand, and occur in the pre-clot stage."[10]

It is not improbable that, under certain circumstances, the normal or natural proteins of the body may be heterologized, and thus acquire antigenic properties and induce sensitization of the individual to the products of dissociation of his own tissues. Batty Shaw had suggested that possibly the progressive course of certain diseases, such as chronic interstitial nephritis, may be due to such a condition. Whether this be so or not, must, for the present at least, be considered to be *sub judice*.[11]

In a certain percentage of cases repeated intravenous injections of salvarsan have been accompanied by a constitutional reaction characterized by dyspnea, throbbing in the head, and collapse. The similarity of this reaction to the acute anaphylactic phenomenon is striking. That such a drug, containing as it does no nitrogen radicles, should possess the property of sensitizing the individual to itself, or of acting as an antigen in the anaphylactic reaction, does not seem possible if our ideas regarding the essential proteid[12] nature of antigens be correct. A similar phenomenon is, moreover, noted by the use of such a simple drug as iodine in Lugol's solution.

Experiments by Swift and Ellis, Wolff-Eisner, and others, have suggested a manner in which these chemicals may perhaps act in inducing true anaphylactic. It has been found impossible to employ salvarsan as an antigen in anaphylactic experiments in the guinea pig. It is possible, however, under suitable conditions, to sensitize guinea pigs against a mixture of salvarsan and guinea pig's serum in such a way that a second injection of a similar mixture proves fatal. The inference drawn is that, as a result of the intravenous injection of salvarsan, certain protein constituents of the blood are so

[10] It should be mentioned in passing that, although, under the conditions of rapid clotting, employed by DeKruif, such toxic properties were frequently developed, in clinical experience they occur but infrequently.

[11] For a more extensive presentation of this subject, the reader is referred to articles by Longcope and Boughton.

[12] As mentioned elsewhere Danysz believes all colloids to act as antigens.

altered that they act as a protein antigen, and thus induce the development of a specific antibody. As a result of the presence of this antibody the animal is rendered sensitive to the subsequent introduction or presence of the same altered protein in the tissues.

Constitutional Manifestations of Infection and Reaction

The clinical symptoms and signs of infectious disease depend upon:

a. Rate of growth of bacteria and presence or absence of essential toxin production on their part.

b. The physical characteristics of bacteria with especial reference to thickness of ectoplasm and capsule formation.

c. The location and function of the tissues in which the infecting bacteria find the most favorable medium for their growth.

d. The susceptibility of special tissues to intoxication.

e. Production or nonproduction of decomposition products from tissues destroyed by bacteria.

f. Length of incubation period, which in turn depends upon: Degree of hypersensitiveness and tolerance of the tissues to the bacteria-protein and factors under "a" and "b."

g. Type[13] or predominant character of reaction employed by the tissues: 1. Antibody elaboration; 2. Cellular phagocytosis.

When viable bacteria gain entrance into the tissues of the body, any one of several phenomena may be exhibited.

1. The protective powers of the body may be sufficient to at once overcome the invader and neutralize its poisons, so that no evidence of infection and no manifestations of either focal or constitutional reaction are provoked.

2. There may occur no reaction whatever on the part of the tissues of the invaded host. In this event the latter is, more or less, rapidly overwhelmed as a result of the rapid and extensive dissemination of the microorganisms and their

[13]The type of reaction employed by the tissues is largely determined by relative solubility of the bacterial cytoplasm as under "b".

toxic products throughout the body. Death in such cases soon supervenes. The fatal termination is preceded by a stage of gradual collapse characterized by mental lethargy, lowering of blood pressure, increase in the pulse rate, drop in temperature, and leucopenia. In addition to these characteristic symptoms, there is usually noted a definite symptom-complex marked by weakness and malaise, headache and generalized pain, and anorexia. In addition, focal signs of tissue injuries, as in gangrene and malignant edema, are exhibited. The length of time intervening between infection of the tissues and death of the individual depends upon the importance of the tissue attacked and the rate of growth of the infecting microorganism.

In this combination of symptoms and signs we note the usual effect of infection without reaction on the part of the body tissues. Death in such cases is due to two factors: (1) a loss of normal functioning capacity on the part of parenchymatous tissues, and (2) a direct toxic action upon susceptible cells, chiefly those of the central nervous system.

3. Following infection by pathogenic microorganisms, there usually occurs a longer or shorter period, usually lasting from one to two days to an equal number of weeks, known as the incubation period; a period during which the invader continues to multiply and during which there is at best an inadequate reaction on the part of the host. The incubation period terminates with, either the onset of symptoms of dissolution as described in section two, or with the commencement of the reaction on the part of the body. The evidence of this onset of reaction is clinically exhibited by pyrexia, rapid pulse rate, and usually by an increase in white blood cells—leucocytosis.

The commencement of manifestations of intoxication and reactive phenomena is due to the development of the first order proteolytic antibody as the result of which the individual bacterial cell becomes not merely a nonirritant foreign substance discharging a minimal amount of toxins and liberating a small amount of poisonous products, dissociated from the tissues as a result of the biologic activities of the

bacterium, but each individual bacterium which is brought in contact with body fluid is at once so acted upon by this antibody (anaphylactin) that an irritant (anaphylatoxin) is set free. There follow symptoms of irritation of various tissues, notably the blood vessels, and generalized increased metabolism as indicated by elevation of temperature, acceleration of respiration and pulse rate, and increase in total nitrogen elimination. The phagocytes, both fixed tissue cells and polymorphonuclear leucocytes are stimulated to attack the irritant particles. In consequence of this increased demand of leucocytes, the myelogenous tissues, unless they themselves be overwhelmed, increase the manufacture and discharge of leucocytes into the blood. Thus, not only does a leucocytosis develop but it is found, if a differential count of the lucocytes according to the method of Arneth be made, that less mature cells make their appearance in the blood stream.

Should the demand for leucocytes, arising from the local accumulation and destruction of the same, be increased beyond the capacity of the myelogenous tissues to supply such cells, leucopenia ensues. It is thus evident that in the course of an acute infection, such as streptococcus cellulitis, pneumonia, appendicitis, etc., diminution in the number of leucocytes must be interpreted as indicating, either that the demand for cells has lessened, and that, therefore, a more or less immediate recovery of the individual from the disease is likely, or that the capacity of the myelogenous tissue for the production of cells has been overtaxed. When exhaustion of the leucocyte producing function has thus been brought about an early fatal termination of the disease may be expected. In the latter event the examination of the blood shows an undue proportion of immature leucocytes.

Isaeff was the first to draw attention to the fact that if, prior to the onset of symptoms of reaction to infection, a leucocytosis was induced, by means of the introduction of various substances such as nuclein, salt solution, or normal serum, parenterally into the tissues, the subsequent course of the

disease was shortened. The explanation of the usefulness of this reaction is comparatively readily understood, since the simultaneous activity of a large number of phagocytes may frequently be the determining factor in accomplishing the destruction of the infecting bacteria.

4. Still another series of phenomena may occur which is exemplified by diseases which are termed chronic inflammations, such as tuberculosis, syphilis and blastomycosis, as well as other essentially local diseases such as furunculosis, acne, and gonorrheal urethritis. If as a result of a natural or inherent slow rate of proliferation on the part of the infecting microorganisms, or on account of their localization in such a place, or in such a manner, that the natural production of proteolytic antibodies is inadequately stimulated, it is noted that the incubation period is considerably prolonged or that the onset of reaction is exhibited only locally.

If the development of the sensitizing first order antibody (anaphylactin) proceed sufficiently slowly or be sufficiently long delayed a state develops analogous to, or identical with, that found in the immune or tolerant animal. A second antisubstance appears which is potent to render the product of the reaction between anaphylactic antibody and protein antigen, innocuous and nonirritating. As a result the infected individual does not show the same symptoms of intoxication nor are the leucocytes stimulated to attack the bacterial cells. Leucocytosis is not exhibited. Although there is proof that the body fluids can, under certain conditions, destroy certain types of bacterial and animal parasites, this effect is more rapidly and economically brought about, if the body cells, more particularly the polymorphonuclear leucocytes, are stimulated to activity. We note that, in infections in which leucocytic accumulation is not a marked feature of the reactive process, the eradication of the invader from the tissues proceeds comparatively slowly. For instance, at no time during the course of a syphilitic infection do evidences of constitutional disturbances attain the severity of those which characterize pneumococcus infection. Nevertheless, the ultimate outcome in syphilis is more often fatal than in pneu-

monia, unless specific chemotherapy, in the treatment of the former disease, be employed.

We thus note that there is a group of infectious diseases characterized by a prolonged clinical course, moderate or slight pyrexia, and accompanied with but little increase in pulse rate, in which leucocytosis is either absent or moderate in amount. Such affections are called chronic. The chief primary factor determining chronicity is the slow growth of the infecting bacteria and protection of their cytoplasm from the action of body fluids. The most important property determining such protection is capsule formation. Especially are these phenomena noted if the bacterial accumulations be so situated, anatomically, that their intimate contact with body fluid is more or less precluded. As the result of either or both of these circumstances, tolerance to the bacterio-protein is developed almost as rapidly as is hypersensitiveness. Cellular reaction (phagocytosis) is, therefore, stimulated but little.

5. If, for any reason, reaction against a rapidly proliferating microorganism be prolonged for any considerable length of time, the whole body may become inundated with bacterial units. If the anaphylactic body be produced in sufficient quantities the bacterial cytoplasm may form the substrate from which a quantity of irritant (endotoxins of older writers) sufficient to overwhelm the tissues, is produced. Thus we find that other factors being constant, the longer the termination of the incubation period is delayed, the more severe are the clinical symptoms of disease.

The Rôle of Anaphylaxis in Resistance to Infection (Vaughan's Conception)

Vaughan[14] performed a series of experiments[15] which were carried out with bacterial cell bodies grown in large quantities. The bacterial substance was washed with salt solution

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[14]Vaughan: Proc. Instit. Med., Chicago, 1920, iii, 39.

[15]An interesting phenomenon was noted in carrying out these experiments. It was found necessary to wear masks during the grinding process, and even when this was done the person who ground typhoid bacillus for the first time suffered within from four to six hours a severe chill followed by a temperature which ran as high as 106° F.

and then extracted for three days with absolute alcohol, and for four days with ether. This treatment left a white powder as all the coloring matter was removed by the extraction. The extracted cell substance was ground first in porcelain, and then in agate mortars. Under the microscope the bacterial substance had the same appearance as in fresh specimens.

The dead cellular substance injured normal animals, and "strange to say it harmed in inverse proportion to the infectivity of the living organism." For instance, prodigiosus, a nonpathogenic bacterium, killed guinea pigs when injected into the abdominal cavity in the proportion of one part of bacterial extract to two or three million parts of body weight. In no amount did the tubercle bacillus kill fresh animals. Vaughan found that the dead substance when injected into animals produced the same lesions that follow inoculation with the living bacillus. He, therefore, concludes that it is not the growth and multiplication of bacillus in the animal body that cause the symptoms and lesions of the disease. In an earlier section of this chapter the author has discussed the factors which may be instrumental in determining a specific type of reaction.

Vaughan noted in his experiments that the effects of the dead substance upon animals are inversely proportional to the infectivity of the living organism. He explains this apparent paradox as follows: Bacteria, such as the prodigiosus, are, he believes, nonpathogenic because the body cells are already sensitized to this organism. As a result, small doses of bacteria are destroyed as soon as they enter the body and infection is prevented, while if larger doses of the dead bacterial substance are injected the normal fluids of the body immediately split up the cells of the prodigiosus, and if the quantity injected be sufficient the toxin split product kills the animal. Vaughan sums up the facts upon which depend the infectivity of bacilli or other viruses as follows:

"1. Will it grow in the animal body? If it will not grow in the animal body, it cannot cause infection. A given organism must grow and multiply in the body in order to be

infectious, and in doing so it must be able to feed on the substance of the body. If it is not able to do this, it cannot be infectious to that animal.

"2. Whether a given microorganism is pathogenic to a given animal or not, will depend on whether the fluids[16] of the body kill that organism as soon as it is introduced into the body. If this does not happen, and if these two conditions are favorable, the microorganism grows and multiplies in the body and causes infection."

When a guinea pig receives an intraperitoneal injection of typhoid bacilli, the following phenomena are noted:

"For some hours there is no recognizable difference between the inoculated animal and his fellows. Their behavior is the same. Then, rather suddenly, something goes wrong with the inoculated animal. It begins to shiver; its coat gets rough; it huddles up among its fellows; it goes off its food; something is wrong. For a while its temperature may be above the normal, but in a short time it begins to fall and continues to do so until the animal dies. At necropsy one finds an exudative hemorrhagic peritonitis. If something like the minimum fatal dose has been administered, it will be about twelve hours before the animal shows any change. This is the period of incubation, and it occurs in every infectious disease. During this time, the bacilli multiply in the body. They are feeding on body substances. They are converting guinea-pig substance into typhoid substance, and the process is a synthetic one. They are taking relatively simple bodies, probably only amino acids, and building them into typhoid protein. During the period of incubation of an infectious disease, the invading organism furnishes the ferments and the body of the host supplies the substrate or food, and the processes are synthetical. Simple bodies are built into more complex ones. There is no poison set free, there are no lesions, and there are no symptoms. Therefore, it is not directly the

[16]It is to be noted that Vaughan's views in this respect are practically identical with those conceived by von Pirquet. The author agrees with Vaughan's conception, but believes that it is not so much the body fluids, as the stimulation of the phagocytic activity of the tissue cells which determines protection of the hypersensitive individual from infection.

growth and multiplication of bacteria in the body that cause the symptoms and lesions of the infectious diseases.'' Vaughan explains the usefulness of prophylactic vaccinating against typhoid fever in the following way,—''We vaccinate against typhoid fever by taking the dead bacillus and injecting it into the individual three or four times at intervals. In so doing we are training the body cells to digest the typhoid protein and subsequently, when the man drinks water that contains typhoid bacilli, as soon as the first organisms get into the body they are split up and destroyed, and the man escapes typhoid fever.''

Vaughan believes the tissue changes, during infectious disease, to be as follows: The invading organisms multiply in the body until the body cells become sensitized and pour out a secretion that splits up and destroys the invading organisms. During the active stage of an infectious disease the body cells supply the ferment, the invading organisms furnish the substrate or food; the processes are analytic; complex bodies are split into simple ones; a poison is set free, and the symptoms and lesions of the disease develop.

Vaughan (1920) and his associates have made many attempts to induce immunity in animals with his protein poison. They have found, and their observations have been confirmed by others, that a certain degree of tolerance may be established by repeated doses of this substance. Normal guinea pigs or rabbits that have been treated with successive doses of the protein poison may be able to withstand without harm, two or three times the original fatal dose. If such animals are inoculated with the living organism it requires more of the culture to kill these animals than it does to kill fresh animals. Vaughan interprets this type of experiment as indicating that increased tolerance to the protein poison enables an animal to bear at least one fatal dose of the living organism. It must be pointed out that another explanation may be offered, namely, that such animals are in fact rendered hypersensitive in consequence of unaltered protein contained in the poisonous material, and protection from injec-

tion of such animals is, in fact, due to a development of hypersensitive condition with consequent immediate reaction following the entrance of viable bacteria into the tissues.

Factors Determining Relative Susceptibility to Infection

It is apparent that there must be factors, other than simple exposure to pathogenic microorganisms, concerned in the institution of infection. An analysis of these contributing factors is of value since we are thereby enabled to indicate the proper means for the maintenance of health.

To return to our definition of infection: we noted that two conditions must prevail in order that infection may occur. First, it is necessary that the microorganism be enabled to gain entrance to the body tissues, and, secondly, the site of their localization must present conditions adapted to the cultural requirements of the invading germs, so that after having obtained a foothold they may be permitted to proliferate.

The initiation of infection depends, in part, upon the bacterium gaining entrance to the tissues and, in part, upon the properties of the tissues themselves. Thus, we note that even though bacteria are of a species which is capable of continued proliferation in the animal tissues, the resistance of the tissues to the invader may be such that unless a considerable number of bacteria are introduced, they are instantly destroyed. This fact is in all probability explained by the phenomenon of absorption. For instance we may assume that the available antibody, using this term in its broadest sense, in a given focus, represents a total of 100 units. If, into the tissues of such an animal, there be introduced a suspension of bacteria representing 10 units, all will be destroyed; whereas if the dose be increased to 150 units, absorption of the antibody may take place without the death of a single organism.

In order that infection may occur, the infecting microorganisms must be capable of maintaining their vitality and proliferating under such circumstances, as regards temperature, moisture, oxygen supply and quality of foodstuffs, as are found in

the animal employed. For this reason the majority of bacteria and protozoa are incapable of infecting the warm blooded animals, including man, unless as a result of trauma, excessive heat or cold, the normal conditions as regards these factors are disturbed. Thus true infection by anaerobes, such as the B. aerogenes capsulatus, and the bacillus of malignant edema cannot occur unless the tissues are injured by trauma. Growth of bacteria of this type takes place, only, in those tissues whose vitality and consequent oxygenation has been destroyed.

To sum up, in order that infection may be accomplished, microorganisms must possess sufficient *virulence* and be introduced in sufficiently large numbers that the natural protective properties of the focus of introduction may be neutralized.

This aspect of the subject of susceptibility to infection is, however, of comparatively little interest except insofar as the anaerobes are concerned. The surgeon, as well as all other practicing physicians, is interested in those factors which render one individual more or less susceptible to infection than his fellows, or than himself, under different conditions of health and environment.

Insofar as the individual is concerned the resistance against, or liability to, infection depends upon one or other of several factors, the most important of these being the immune body content of his body fluid and tissues. This is influenced by a variety of circumstances and may be natural depending either upon an absence of sensitive cells, or inherited from parents (natural immunity). On the other hand previous attacks by the same or closely allied microorganisms may have stimulated antibody production (active immunization), similarly subinfection or therapeutic inoculation with vaccines may have brought about a like result; again too, immunity may be exalted for a short period by means of the introduction of serum from a highly immune animal (passive immunization). Since the greater proportion of available antibodies are present in the blood and lymph fluids, and since the most actively phagocytic cells are those of the blood, protection from infection depends upon the maintenance of an adequate blood supply, particularly to those parts of the body subject to insult.

Two nonspecific protecting properties are liable to depression or decrease under certain conditions of exhaustion and exposure. These are phagocytic activity of the body cells and alexin. These factors which affect the susceptibility of the individual are adversely affected by:

a. Malnutrition (starvation or dehydration).

b. Injury (loss of blood and shock).

c. Exhaustion from overstrain, physical or mental.

d. Chilling of the body or excessive heat.

e. Certain chronic diseases.

It is as a result of the action of one or more of these depressing influences that terminal or terminating infections occur, e.g., bronchopneumonia and dysenteric affections.

Equally as important in their effects upon constitutional susceptibility to infection as the above influences, are those which affect the local resisting power of tissues. In this respect we note that there are certain inherently susceptible tissues. Thus we note that those areas of the body best supplied by blood vessels, especially if such be not "terminal" in nature, are much less susceptible to infection than other parts of the body. Recent experiments have shown that certain groups of vessels are much more liable to pressor influences as the result of irritation, and that those areas supplied by such vessels, as, for instance, the anterior aspect of the lower leg, are peculiarly liable to infection. This suggests a second and almost equally important factor, namely, the production of relative local anemia as the result of nervous influences induced by trauma, draughts of cold air, etc. The result of either the natural absence of adequate blood supply or an acquired ischemia, is the same; viz., a diminution in the amount of available protective serum antibodies and blood cells, and a lessened vitality of the fixed tissue cells themselves. Similar effects are noted, as the result of either arterial or venous obstruction, whether these be due to intravascular occlusion (thrombosis, endarteritis, etc.) or extravascular pressure as in certain forms of edema accompanying frost bites and occurring in cellulitis from other causes.

Injury, either mechanical, chemical or physical, may lower the resistance of a part in one or more of several ways:

1. Direct destruction of tissue cells and occlusion of vessels. Severe contusions, burns, frost bites.

2. Pressor stimulation.

3. Stimulation of extravascular accumulation of body fluid and consequent slowing of the blood stream.

Impairment of sensory nerve supply of a part, as, for instance, the fingers and toes in leprosy and syringomyelia, and as recently seen exemplified by the large number of nerve injuries consequent upon gunshot wounds, deprives the tissues of the natural protection of sensation and consequent reflex or purposeful removal of the part from such source of injury as intense heat and cutting and bruising instruments.

Not infrequently the normal protection of the part, such as the skin and adequate vascular supply, is interfered with as the result of the presence of other forms of disease such as tumor, and previous inflammations, either active or healed. Under such conditions the tissues are rendered more susceptible to infection.

Relapses.—Relapses during the course of disease may be due to one or all of the following causes:

1 Depression of alexin.

2. Depression of first order antibody—anaphylactin or allergin.

3. Destruction of leucocytes and inhibition or exhaustion of the myelogenous function.

4. Depression of phagocytosis due to exaltation of second order antibody and exhibition of tolerant state.

Of these four possible reasons for relapse, wearing out of the myelogenous tissues and overtaxing of the tissue cells, responsible for the elaboration of the antibodies, are doubtless the more usual causes. As a matter of fact, it is rarely that the gradual progress toward cure is interrupted unless the tissues are subject to some added strain, such as exposure to undue or too prolonged cold, gastrointestinal intoxication, or some intercurrent new type of infection.

CHAPTER XXV

APPLICATION OF IMMUNITY PRINCIPLES TO THE PREVENTION AND TREATMENT OF DISEASE

The fact that infection by various pathogenic agents frequently results in protection of the individual from subsequent contraction of the same disease, has been recognized for centuries. The first record of any attempt to make use of such observations for therapeutic purposes dates back about two hundred years. At this time it was the custom among the Turks to inoculate their children with the contents of small-pox pustules in order that by this means they might be protected from the ravages of more severe epidemics. This method was introduced into England by Lady Montague. Since, however, such a procedure not infrequently resulted in very severe "takes," its popularity was not maintained. As an outcome of this more or less hazardous procedure, the use of cow-pox virus, as introduced in England by Jenner in 1796, developed. The subsequent development of immuno-therapeutics up to the stage at which we meet it today dates from the time of Pasteur's epoch making researches (1878-1887) upon anthrax, rabies, and chicken cholera.

Since it is possible to stimulate, by means of inoculation, the production of potent immune bodies—active immunization—and, also, to confer a certain degree of immunity—passive immunization—through the introduction into the animal tissues of antibodies derived from an actively immunized animal, both of these methods have been employed in the prophylaxis and treatment of disease.

Serum Therapy

The first attempt to confer passive immunization upon an animal by means of the introduction of blood serum from an-

other animal, was that of Salmon and Theobald Smith[1] in 1885. These observers were successful in procuring a protective serum against hog cholera, which proved of very considerable value. But little enthusiasm, however, for the new method was aroused until the results obtained by von Behring and Kitasato, and by Roux and Yersin in the production of of a serum which showed itself to be potent to influence favorably the clinical course of diphtheria, were published. In 1893 von Behring first introduced his antitoxin for use in human diphtheria. The satisfactory result of this form of therapeutics is a matter of common knowledge.

If the medical and lay world had previously been apathetic, they immediately became correspondingly overenthusiastic and were even sanguine that the millennium had arrived, at least insofar as the control of acute infectious disease was concerned. Numerous investigators directed their efforts towards the development of sera which it was hoped might be used against all the known pathogenic bacteria and against numerous toxins, including those of bacterial, vegetable, and reptilian origin.

For a time the most extravagant claims were forthcoming with reference to the curative properties of this or that serum. Unfortunately, however, it was, before long, evident that if it be possible to produce curative or prophylactic sera against the majority of bacterial and protozoal diseases, this desideratum has not yet been attained, and will be, if ever, only when continued investigation and experiment have taught us more of the facts regarding the nature, mode of production, and action of immune bodies.

In general it may be stated that no serum has been produced which has proved of such striking usefulness as antidiphtheritic serum, although those prepared against the toxins of the tetanus bacillus and various snake venoms are, under favorable conditions, very valuable. In addition to antitoxic sera against the toxins of B. diphtheriae, B. tetani, and the snake

[1]Salmon and Theobald Smith: Rept. of Comc. of Agric. Washington 1885-1886.

poisons, sera have been developed of considerable potency against the B. botulinus,[2] and the B. pyocyaneus.[3] It will be noted that the foregoing sera are essentially antitoxic and not bactericidal. It is against the various toxins that sera are most readily produced.

The report of an interesting series of experiments was published in 1921 by Huntoon,[4] the results of which may be very far reaching. Already clinical application of the principle discovered has been made, and favorable results in the treatment of lobar pneumonia reported.[5]

The aim of Huntoon's work was to prepare a serum-free solution of antibody. The method employed by Huntoon is as follows:

"Horses are injected at regular intervals with emulsions of Types I, II, and III pneumococci. The serum after a number of injections develops protective antibodies. To obtain a serum which protects mice against 1,000,000 lethal doses or more of Type I is readily accomplished. It is more difficult to obtain as high a protective power for Type II, and against Type III a serum can seldom be obtained which protects against more than 100,000 fatal doses.

"To this serum is added an equal volume of a heavy emulsion of living pneumococci Types I, II, and III. The mixture is placed at 37° C. for one hour or 20° C. for twelve hours, and then centrifuged. The sediment is washed with salt solution to rid it of horse serum. The washed sediment is emulsified in salt solution containing 0.25 per cent sodium bicarbonate and heated to 55° C. for thirty minutes to one hour. This causes dissociation of the pneumococcus (antigen) and antibody. The mixture is centrifuged, and the supernatant fluid removed, chilled, recentrifuged, and finally filtered through a filter candle. The final solution, which contains only 0.035 mg. of nitrogen per cubic centimeter, is in many lots able to protect mice against as many fatal doses of pneu-

[2]Kemper: Ztschr. f. Hyg., 1897.
[3]Wassermann: Ztschr. f. Hyg., xxii, 1896.
[4]Huntoon: Jour. Immunol., 1921, vi, 117.
[5]Cecil and Larsen: Jour. Am. Med. Assn., July 29, 1922, lxxix, 343.

mococcus Types I, II, and III as the original serum from which it was made."

In this manner the aqueous solution of specific antibodies is obtained in a practically serum-free state. Protective substances against pneumococcus Types I, II, and III are obtained in great concentration: the disadvantages in the use of serum due to the serum-proteins are eliminated. As pointed out by Cecil and Larsen this solution contains, in addition to antibody, a small amount of pneumococcus protein which may conceivably act in the capacity of a vaccine and, therefore, produce a certain amount of active immunity.

Cecil and Larsen employed monkeys in their experimental work.

"The most striking results were observed in experimental pneumococcus Type I pneumonia. Following the injection of antibody, pneumococci immediately disappeared from the blood, and the animal made a rapid recovery. When antibody was administered to monkeys that had been inoculated with lethal doses of pneumococcus Type II, the results were not so striking. A certain number, however, were saved by this mode of treatment. In the case of experimental Type III pneumonia, no benefit whatever could be obtained by treating the infected monkeys with antibody solution. Since the antibody solution displays its highest protective power in mice against pneumococcus Type I, next highest against pneumococcus Type II and least against pneumococcus Type III, it would appear that the beneficial effect induced by its administration is, in large measure, proportional to the amount of protective substance present."

The experiments performed by Cecil and Larsen were carried out on a very large scale: 834 cases of pneumonia due to pneumococcal infection, and 166 cases due to streptococcus and other infections, were employed in the course of their work. Of the twelve medical wards in the hospital six were placed in each of the two groups; in the one group patients were treated by means of antibody solution, and in the other

group this specific form of treatment was not employed. The accompanying table summarizes their results.

It is evident that pneumococcus antibody solution so prepared is a therapeutic agent of considerable power. The most striking results are obtained in pneumococcus Type I pneumonia; in pneumococcus Type II pneumonia the results are less impressive; and in pneumococcus Type III the antibody solution appears to possess no benefit whatever. In the Type IV pneumonia there has been a considerable difference in the death rate, the reason for which these authors are unable to suggest.

COMPARISON OF DEATH RATE IN TREATED AND CONTROL SERIES

TYPE	ANTIBODY WARDS			CONTROL WARDS		
	CASES	DEATHS	RATE %	CASES	DEATHS	RATE %
Pneumococcus I......	158	21	13.3	162	36	22.2
Pneumococcus II.....	83	23	27.7	67	27	40.3
Pneumococcus III....	73	29	39.7	60	24	40.0
Pneumococcus IV	110	18	16.4	121	29	24.0
Total	424	91	21.4	410	116	28.3
Streptococcus, etc....	48	24	50.0	35	12	34.3
Unclassified..........	36	14	38.8	47	20	42.5

Doses of from 50 to 100 c.c. of the solution have been given once, sometimes twice, and occasionally three times a day. The typical reaction is thus described.

"There is no immediate reaction. From twenty to forty minutes after the injection, the patient begins to shiver and is soon in the midst of a hard chill. The cyanosis and dyspnea become more marked, and the patient often shows extreme anxiety. The chill lasts from fifteen to thirty minutes. At its conclusion, the patient complains of fever, and the temperature may have risen to 106° F. or even to 108° or 109°. In rare cases the temperature may rise to 110°. In one case, the rectal temperature was too high to be recorded on the thermometer. When the thermometer was removed, the bulb was missing, and a careful reading of the mercury column recorded 113.1°! The patient was wildly delirious during this period of hyperpyrexia, but ice packs were followed by a rapid drop, and on the next morning he

showed a normal temperature and made an uncomplicated recovery. * * *

"The high temperature usually persists for only a short time: from thirty to sixty minutes. The rapid fall is accomplished by a profuse perspiration, which often drenches the patient's linen, and which may continue for several hours. The fall may be slight, but is usually extensive, often reaching normal or even subnormal limits. It may be temporary or permanent. It is more likely to be temporary if treatment is started early; permanent if the injection is given when the crisis is about due. In some cases, however, one or two injections appear to abort the infection completely, and the temperature remains normal on the third or fourth day of the disease. * * *

"In three cases, the reaction following injection with antibody appeared to be the immediate cause of death. * * *

"The average number of injections was 3.6 for each patient, and the total amount of antibody administered averaged 225 cubic centimeters for each patient."

Cecil and Larsen are of the opinion that the beneficial effect of the antibody solution is probably not referable to the shock reactions, otherwise an improvement would be expected in pneumococcus Type III, and in streptococcus pneumonia, as well as in other pneumococcus types.

It is not my intention at this time to attempt to analyze the action of the antibody solution; further work is necessary before such an analysis can be made. It must, however, be pointed out that in view of the marked febrile reaction which accompanies introduction of the solution it remains to be proved that the curative effect is specific and not due to exhaustion of tolerant antibodies in the infected individual with consequently more adequate reaction on the part of the tissue cells.

In any event this series of experiments is of very considerable interest, and probably, as indicated above, will be of far-reaching importance.

Production and Preparation of Antitoxic Sera

Since the introduction of heterologous proteins parenterally into the tissues is very frequently followed by evidence of intoxication, and since the serum from certain animals has, naturally, the property of producing hemolysis of human erythrocytes, it is essential, in the employment of serum therapy, that an animal be chosen for immunization purposes whose serum is as innocuous to human beings as possible.

The animals best fitted for purposes of antibody production for use in the passive immunization of man are, the horse, ram, goat, and rabbit. Of these the horse is by far the most serviceable, the more so since the size of the animal makes it possible to procure a large amount of blood without injury to the donor.

Method of Production of Antitoxic Sera.—When the tetanus bacillus is grown in a suitable medium—neutral or weakly alkaline broth containing a minimal amount of carbohydrate—two toxins are produced. One, a hemolytic substance, is unimportant; the other, known as tetanospasmin, is the poison responsible for the manifestation of human tetanus. In the production of antitoxic sera against tetanospasmin healthy young horses are employed. The animals are injected with broth in which tetanus bacilli have been growing under anaerobic conditions for six or ten days. The first doses of the toxin are either weakened by means of chemicals, or are treated with twice the neutralizing amount of antitoxin, in which case 3000 units or more of the toxin are injected. Injections are repeated at three to five day intervals until the animals tolerate from 500 to 600 thousand units of toxin.

When the horse is sufficiently highly immunized, it is bled from the jugular vein. The blood is allowed to coagulate and the serum removed after storing for several days in the ice chest. As a rule a germicide—0.5 per cent phenol. or 0.25 to 0.40 per cent tricresol—is added. Horses vary in their immunizing capacity, only about 10 per cent are able to develop high antitoxic potency. If well looked after favorable animals

may be bled at intervals of a month or six weeks over a period of two or more years. Six liters may be procured at each bleeding.

If stored at room temperature serum loses about 20 per cent of its antitoxic activity in one year; if kept in the ice chest (5° C.) this loss is much reduced; but 6 per cent deterioration occurs in the same length of time.

Antitetanic serum is standardized against a standard quantity of tetanotoxin. One unit of the toxin is the smallest amount which will kill a 350 gram guinea pig in four days. In the United States the standard toxin is supplied by the United States Public Health and Marine Hospital Service. The unit of antitoxin is ten times the amount that will protect a 350 gram guinea pig for ninety-six hours against one official dose of the toxin which contains 100 units. The German unit is much larger (about seventy-five times) and is determined by a method which gives less constant results (Wood).

Antitoxic Serum—Treatment of Diphtheria

The signs and symptoms of uncomplicated diphtheria are almost wholly due to the action of the specific diphtheria toxin. The treatment is, therefore, directed to the neutralization of toxin by antitoxin. Once the effect of the toxin has been neutralized in this way, the patient appears to have little difficulty in overcoming the bacilli themselves.

When given subcutaneously or intramuscularly, the maximum concentration of antitoxin in the blood is not reached before from 24 to 72 hours after injection. In severe cases, therefore, it is of value to introduce the antitoxic serum directly into the blood stream. The antitoxin persists in the blood for a number of days. As pointed out by Park, it is owing to misunderstanding of this fact that the employment of multiple doses is due. Observations carried out by this investigator over several years in hospitals in New York City have shown that if the first dose injected be sufficiently large, a single dose is all that is required in any case. On the other

hand, the insufficient first dose cannot be wholly compensated for by later injections. The following table is quoted from Park[6]:

	MILD CASES	EARLY MODERATE	LATE MODERATE AND EARLY SEVERE*	SEVERE AND MALIGNANT*
Infants 10 to 30 lbs. in weight, under 2 years.	Units 2,000 to 3,000	Units 3,000 to 5,000	Units 5,000 to 10,000	Units 7,500 to 10,000
Children 30 to 90 lbs. in weight, under 15 years	3,000 to 4,000	4,000 to 10,000	10,000 to 15,000	10,000 to 15,000
Adults 90 lbs. and over in Wt.	3,000 to 5,000	5,000 to 10,000	10,000 to 20,000	20,000 to 50,000
Method of administration advised.	Intramuscular.	Intramuscular.	Intravenous	Intravenous.

*When given intravenously, the smaller amounts stated.

"In all cases a single dose of the proper amount as indicated in the schedule is recommended." (Park.)

The various brands of antitoxin which are on the market are prepared by a precipitation of soluble globulins, from the sera of immunized horses. When this precipitation is carried out, it is found that the antitoxin content of the serum is removed along with the globulins. When the latter are redissolved it is possible to procure a concentrated serum which minimizes considerably the dangers and discomforts of serum disease. It must not be assumed, however, that such concentrated globulins are absolutely harmless. The same precautions must be employed that are mentioned in Chapter XXIV.

The Prophylactic and Therapeutic Employment of Tetanus Antitoxin

In man tetanus toxin is fixed only by the tissues of the nervous system. When the tissues are infected by the tetanus bacillus, the toxin which is manufactured at the bacterial focus is absorbed by the nerves in the neighborhood and carried, in the nerves themselves and in the perineural lymphatic vessels

[6]Park: Jour. Amer. Med. Assn., Jan. 8, 1921, lxxvi, No. 2.

(Teale and Embleton), along the course of the nerve trunk to the central nervous system. When the spinal cord is reached, the nerve cells in the anterior horns are injured and clinical symptoms of spasm of the muscles which are innervated by these cells are exhibited. Although there can be but little doubt that the majority of the toxin which is discharged from the focus reaches the central nervous system by way of the nerve trunks, it is not improbable that a certain amount is absorbed into the blood stream and is thus carried to the brain or cord.

Whenever a lacerated wound, burn, frostbite or other type of injury which is accompanied by devitalization of tissue is incurred, a favorable nidus is produced in which the tetanus bacillus may grow.

Since it is impossible to determine whether a wound is thus contaminated or not prior to the onset of symptoms, it is advisable in every case of injury of this sort to anticipate the possible onset of intoxication by the introduction of antitoxin in the form of antiserum. As a general rule 750 to 1500 units are injected, the dose being determined by the severity of the wound and its location; i.e., whether in close proximity to larger nerve trunks. In those cases in which necrosis of considerable masses of tissue occur, the antitoxin should be reinjected in the same quantities at weekly intervals until all necrotic tissue has sloughed off. In those cases in which the wounds are in the neighborhood of nerve trunks, particularly if close to the central nervous system as the loin, neck and head, it is advisable that the serum be introduced in the neighborhood of the injury.

Treatment of Tetanus with Serum.—In the majority of cases it is possible to diagnose tetanus before the spasms have become generalized. Since the toxin reaches the central nervous system by way of the nerves, spasms are first exhibited in those muscles whose nerve supply is derived from the part of the cord to which the nerve trunks transmitting the toxin deliver the tetanospasmin. Consequently, in the majority of cases, careful observation of the wounded permits diagnosis of the

disease before the distribution of the toxin has become general. This is not, however, usually possible when wounds affect the muscles of the loin or the tissues of the head and neck. In injuries of this nature the toxin has such a short distance to travel that the symptoms of intoxication are likely to be manifest soon after injury and to become rapidly generalized.

In the treatment of tetanus, in addition to proper surgical cleansing of the focal lesion and the employment of absolute quiet for the patient, antitoxin should always be employed. Antitoxin serum should be administered in large doses. As was pointed out in discussing the treatment of diphtheria by antitoxin, it is advisable to make the first dose, if possible, the total dose.

There exists at the present time a difference of opinion regarding the route of injection which is most favorable. This disagreement, in the author's opinion, is due to the fact that experiments which have dealt with intoxication by tetanus toxin have been confused with infection by the tetanus bacillus. Park and Nicoll found that animals—guinea pigs—which were injected into the hind legs with two fatal doses of toxin and subsequently, 24 hours later, received immune serum, survived in a larger proportion of cases if the serum was injected intrathecally than if introduced into the subcutaneous tissues or the blood stream. Sherrington conducted similar experiments upon monkeys.. He gives the accompanying table of his results.

ROUTE OF INJECTION	TIME BETWEEN GIVING OF TOXIN AND ANTITOXIN	RECOVERIES	DEATHS
Lumbar intrathecal	47–78 hours	14	11
Bulbar intrathecal	47–78 "	13	12
Intravenous	47–78 "	7	18
Intramuscular	47–78 "	3	22
Subcutaneous	47–78 "	2	23
Cerebral subdural, ten cases	47–78 "	0	10

These experiments prove that when tetanus toxin is injected into the muscles, and after an interval an effort is made to neutralize the injected toxin by means of antitoxin, the latter

is more useful if injected into the subdural space. In the ordinary case of tetanus infection in the human being, the prime aim of the surgeon is to prevent the toxin from reaching the central nervous system; for this purpose neutralization of the toxin, close to the point at which it is being produced, should be more useful.

In all severe cases of tetanus, therefore, intrathecal injections of the antitoxin should be made, but never to the exclusion of intramuscular injections in the neighborhood of the bacterial focus. Since the toxin reaches the central nervous system by means of the nerve trunks, an effort should be made to bathe the nerve trunks and nerve endings with the antitoxin containing serum. Since whatever substances are injected into the blood stream, must be assumed to eventually reach the central nervous system, intravenous injections of serum should also be employed.

In the treatment, therefore, of tetanus, large quantities, forty thousand to one hundred thousand units should be injected about the focus. A more moderate dose of fifteen to fifty thousand units should be injected intravenously, and in fulminant cases, two or more intrathecal injections. The amount injected in the spinal canal will depend upon the potency of the serum available and the amount of fluid which can be withdrawn from the piaarachnoid space.

Bactericidal Sera.—Heretofore but indifferent results have been obtained in the employment of bactericidal sera. Although, for several types of antistreptococcic and antipneumococcic sera, extravagant claims have been made, the favorable clinical results following their employment have been very meagre. It does not seem improbable, however, that eventually more desirable effects may be obtained. Improvement in effectiveness will probably depend upon more simple and exact methods of serologic classification of bacteria.

It must be borne in mind that when immune serum is passively transferred to the diseased individual, the specific first (or first and second) order antibodies alone are introduced. We have previously noted that alexin is necessary for the

exhibition of the lytic activity of the specific antibodies and also that in many instances the phagocytic activity of the leucocytes is essential if bacteria are to be destroyed.

Were it possible to transfer active serum (i.e., containing alexin) it is probable that more satisfactory results would be obtained. Blood transfusion from convalescents to infected persons is a method which theoretically, at least, promises much in this direction. There are, unfortunately, obvious difficulties in the way of such a procedure so that heretofore the reports available are meagre. Unger's observations indicate that blood transfused without the addition of sodium citrate is more effective for this purpose than is blood to which this salt has been added.

Antistreptococcus Serum.—Stimulated by the strikingly favorable results which attended the use of antidiphtheritic serum, von Behring attempted to procure serum which might be of service in combating streptococcus infection. The results obtained by this eminent investigator were of little value, nor have subsequent efforts in this direction been crowned by anything approaching a signal success.

Many sera have been produced which show bacteriotropic activity *in vitro* and which have a moderate protective potency; or five different brands of commercial sera on the market, Weaver and Tunnicliff[7] found four to demonstrate a certain degree of activity in these directions.

The best method of inducing immune body production, so far employed, is by the injection of bacteria of varying degrees of virulence obtained from different sources. In this way a polyvalent serum is produced.

Although, as stated above, but relatively little can be expected of any of the sera so far prepared, its use in large doses —100 to 200 c.c.—repeated every twelve hours, may well be employed in all cases in which the prognosis, without some such aid, is obviously grave. If proper precautions (page 247) are employed, intravenous injection of the serum is indicated in such cases.

[7]Weaver and Tunnicliff: Jour. Inf. Dis., 1911, ix, 130.

Antistreptococcus serum, as well as other bactericidal sera, loses its activity (alexin) when stored; since, however, it is possible to reactivate it by the addition of fresh normal serum of a suitable animal, it is likely that a similar reactivation occurs *in vivo.*

Antipneumococcus Serum

The most important contribution to the subject of specific treatment of the pneumonias is that of Cole, at the Rockefeller Hospital in New York. He has divided pneumonias into four groups, dependent upon specific types of pneumococci. The differentiation of the infecting microorganism in each individual case is determined by what he has called the "mouse method."[8]

Up to the present a useful serum has been obtained for the treatment of acute lobar pneumonia due to pneumococcus Type I. The serum is injected intravenously and as soon as possible after the type has been determined. Cole gives the following outline for employment of the serum:

"The amount of serum to be injected at the first dose is from 90 to 100 c.c. and this dose should be repeated every eight hours until the fall of temperature and amelioration of symptoms indicate that the infection has been overcome. The serum injected should be at body temperature, and it should be injected very slowly. The total amount required in the average case is from 200 to 300 c.c., though in severe cases, treated late in the disease, it may be necessary to employ much larger amounts, even as much as 1,000 c.c."

Antimeningococcus Serum.—Flexner and Lewis have been successful in perfecting a serum first suggested by Jochmann, which has been proved to be of great value in the treatment of cerebrospinal meningitis if injected directly into the subarachnoid cavity. The effect of the introduction of the serum in

[8]The sputum is washed in salt solution and a small quantity injected into the peritoneal cavity of a mouse. Twelve hours later the mouse is killed, when a practically pure culture of pneumococci is usually obtained. The suspension of pneumococcus, washed free from cells and serum, is then tested by agglutination against each of the four types of antisera. In this way a differential diagnosis is rapidly made.

this way is, according to Flexner's views, threefold: the bacteria are destroyed by the direct bactericidal properties of the serum, phagocytosis is stimulated, and free toxins are neutralized.

There can be little doubt that the employment of this specific serum has revolutionized the treatment of meningococcic meningitis. The unsatisfactory results which have been reported from time to time have been shown to be due to a lack of potency on the part of the serum used (Blackfan). Either polyvalent, or better, a specific monovalent serum, may be employed, the latter only after the type of meningococcus has been determined.

The serum should be employed as early as possible in the course of the disease and should be injected directly into the spinal canal or cerebral ventricles, or both. It should be injected at frequent intervals and in as large amounts as are safe. It should not be discontinued until after the microorganisms have disappeared from the spinal fluid and the patient's general condition improved.

The amount of serum introduced into the canal depends upon the amount of cerebrospinal fluid which can be withdrawn by lumbar puncture. It should not exceed this amount, except in those cases in which a thick purulent exudate cannot be withdrawn. In such cases only 5 or 10 c.c. of the serum should be introduced. It is advisable to employ the gravity method only for its introduction, otherwise symptoms due to increased cerebrospinal pressure may be encountered.

Bacillary Dysentery

Although the results of serum treatment of dysentery have proved to be of relative value only, it should always be employed, if available. It is of the utmost importance that a differential diagnosis between the two types—Shiga and Flexner—be made, since sera prepared by the immunization of horses against one type is of comparatively little use in the treatment of disease due to infection by the other bacillus.

The serum is employed subcutaneously, and in severe cases, intravenously. According to Flexner, the dose for an adult is 20 c.c., but in severe cases, he advises giving doses of from 50 to 100 c.c. intravenously. If sufficient improvement in the condition of the patient does not follow the first dose, it should be repeated in from twelve to twenty-four hours.

Antigonococcus Serum.—Roger and Torrey[9] have prepared and introduced a serum of some potency in the treatment of gonococcal arthritis and synovitis. Although the serum prepared after their technic contains a moderately high content of recognizable antibodies—agglutinins, precipitins complement binding body, and bactericidal (*in vitro*) bodies, the results obtained in practice are by no means striking.

The serum is prepared by the immunization of rams to different strains of the gonococcus; it is, therefore, a polyvalent serum. Roger and Torrey recommend the employment of 2 c.c. daily.

Other Bactericidal Sera.—In addition to antistreptococcus, antipneumococcus, antigonococcus and antimeningococcus sera, and serum employed in the treatment of dysentery, numerous other sera have been introduced by a host of different observers, with, however, but very little effect upon the course of clinical infections.

Antityphoid serum has been used more or less extensively, and according to Chatemcre, with favorable results. In general, however, this method of treatment has not met with any great favor. Sera have been used in the treatment of plague (Yer sin) and cholera. The results, however, have not been strikingly useful.

Recently Symmers[10] has published a resumé of the recent work on anthrax, in which he recommends that under no circumstances should the anthrax pustule be tampered with in any way. He recommends as the only permissible form of local treatment the injection at the periphery of the pustule of broken doses of antianthrax serum at intervals of four or six

[9] Roger and Torrey: Jour. Am. Med. Assn., 1907, xlix, 918.
[10] Symmers: Ann. of Surg., 1922, lxxv, 663.

hours, each injection not to exceed a total of 10 or 15 c.c. Intravenously the dose of 150 to 200 c.c. of antianthrax serum is injected, this is supplemented by the intravenous injection of 40 c.c. every four or eight hours. He states, "In anthrax septicemia the liberal use of antianthrax serum, if commenced in time, is capable in many cases of sterilizing the blood with astonishing rapidity."

During the last few years the relatively high mortality, especially among the poorer people, from measles has attracted attention to this disease. Owing to the highly contagious condition of children in the early days of prodromal symptoms, it is extremely difficult to prevent exposure, therefore procedures have been undertaken in an effort to render children immune. One of these methods has consisted in purposeful injection of very young infants, since it is known that during the first few months of life infants usually exhibit a natural immunity, and it is hoped that by purposeful injection of the child during this period that a more lasting immunity may be produced. This method is still in the experimental stage. There are obvious objections to its employment.

Another method described by McNeal[11] in which children exposed to measles received intramuscular injections of 5 c.c. of serum obtained from healthy donors between the fifth and ninth days after disappearance of the fever accompanying an attack of measles. In this way sixteen exposed children were injected; twelve remained free from measles and four developed a mild form of the disease. As one child contracted measles two months after successful injection this author remarks that in some cases, at least, the immunity does not persist longer than sixty days. As McNeal points out, in institutions in which large numbers of frail children are intimately associated, the procedure may prove to be of great value.

The recent valuable and interesting work which has been carried out by Doctor George F. Dick[11a] and his wife upon the infecting microorganism in scarlet fever, appears to show that

[11] McNeal: Jour. Am. Med. Assn., 1922, lxxxvii, 340.

[11a] Dick, G. F., and Dick. Gladys H.; Scarlet Fever Toxin in Preventive Immunization, Jour. Am. Med. Assn., February, 1924, xxcii, p. 544.

the symptoms of scarlet fever are, in fact, due to intoxication by the toxins produced by a hemolytic streptococcus. They have further shown that when persons with positive skin tests for susceptibility to scarlet fever are injected with suitable quantities of the toxic filtrate, they may develop scarlatinal rash with nausea, vomiting, rise of temperature and general malaise. These symptoms appear within a few hours after the injection and disappear within forty-eight hours. Following this reaction, the skin test is negative, or only slightly positive. The short interval between the injection and the beginning of the reaction, compared with the incubation period of about forty-eight hours described in experimental scarlet fever, and the more rapid disappearance of the symptoms, indicate that the effect is produced by a soluble toxic substance rather than by a filterable virus. The resistance to heat at temperatures ordinarily employed to kill bacteria is further evidence that we are dealing with a toxin.

The similarity of the symptoms produced by the filtrate to those of scarlet fever, and the resulting modification of the skin test, indicate the production of some degree of active immunity to scarlet fever.

The neutralization of the toxic substance in the filtrate by the blood serum of a person who had received injections of the filtrate indicates that the toxic substance is a true toxin, capable of forming an antitoxin.

Prophylactic and Therapeutic Employment of Bacterioproteins (Vaccines) and Pollen Extracts

It is possible by means of vaccine[12] or protein extract injections to induce one or other of the following alterations in the immunologic state of the individual.

[12]Wright defines a bacterial vaccine as follows:—"Bacterial vaccines are sterilized and enumerated suspensions of bacteria which furnish, when they dissolve in the body, substances which stimulate the healthy tissues to the production of specific bacteriotropic substances (or antibodies) which fasten upon and directly or indirectly contribute to the destruction of the corresponding bacteria." The term vaccine is employed since Pasteur, in appreciation of the contribution to humanity made by Jenner by the latter's introduction of vaccinia virus in the prophylaxis of smallpox, applied the term vaccination to the prophylactic immunization of individuals against rabies.

1. The tissues, if normal in their relationship to the protein employed, may be rendered hypersensitive so that they become subject to exhibition of the allergic phenomenon when the same antigen is later introduced into the tissues.

The prophylactic employment of vaccinia virus, and of bacterial extracts or suspensions, is made use of, chiefly, for this purpose.

2. If the tissues be hypersensitive, tolerance may be induced so that they are no longer subject to injury as a result of contact with the foreign protein to which they may be exposed.

The employment of pollen extracts, horse serum, and certain foodstuffs—in the treatment of individuals suffering from hay fever, asthma, eczema, and other diseases, the manifestations of which depend upon protein anaphylaxis—is used with a view to inducing tolerance to the antigen.

3. Tolerance, if present, may be depressed in order that tissue hypersensitiveness may be exhibited, and inflammatory reactions induced at foci of bacterial accumulation.

Vaccines are employed in the treatment of such diseases as furunculosis, localized tuberculosis, and other infections characterized by a chronic clinical course, as a rule, with a view to inducing such focal reactions.

4. Desensitization may be accomplished if the tissues be hypersensitive.

Desensitization is employed chiefly in order that individuals who are hypersensitive to horse serum may be treated by means of serum therapy. In occasional cases, also, severe rapidly progressive inflammatory reactions may be temporarily controlled by exhaustion of the anaphylactic (first order) antibodies, and the focal reaction, thereby, inhibited. Similarly, also, in the treatment of hay fever (pollenosis), if the patient does not come under observation sufficiently early in the year to gradually induce tolerance, desensitization may, sometimes, be employed with benefit.

It is thus seen that in the employment of vaccines or bacterio-proteins for therapeutic purposes, we are, to say the least, handling a two-edged sword. In order, therefore, that favor-

able results may follow injections of antigenic substances, it is necessary that the physician know, relatively at least, the degree of sensitiveness of the individual to be treated and have a clear conception of the alterations in the tissue and serum reactions which he wishes to induce.

Protection from infection, on the part of the individual, depends, almost entirely, upon hypersensitiveness to the bacterial protein. If the tissues of the individual have not been previously exposed to the presence of the specific bacterial protein, the entrance parenterally into the tissues of viable pathogenic bacterial cell bodies, is followed by no exhibition of reaction on the part of the tissues. In consequence, the bacteria are permitted to proliferate unhindered by tissue reaction. The invasive power of the infecting bacterium, under such conditions will depend upon its adaptability for growth under the conditions which maintain in the tissues of the host, and upon its inherent rate of multiplication.

If the microorganism finds in the tissue, into which it has gained entrance, conditions as regards temperature, moisture, oxygen supply, and foodstuffs, suitable, and its natural rate of reproduction be rapid, it will spread widely throughout the tissues of the body without reaction on the part of the tissues taking place. Thus, we find that the Bacillus typhosus entering the tissues of the intestinal tract, finds a suitable medium for its growth, and thence rapidly invades practically the whole body. This general invasion takes place even though the infected individual experiences little, or no, symptoms of intoxication, or tissue irritation; nor do the tissues make any effort to destroy the invader. It is only after the lapse of a certain number of days (six to fourteen) that substances are produced, which have the capacity for reacting with the bacterial cell. When this occurs the patient immediately appears ill, and symptoms of tissue irritation are manifest.

The course of the disease from this time on, is, in a sense, a battle between the serum bodies, assisted by cellular activity, and the bacteria. Unless the patient succumb to an extreme production of toxic substance, or unless an accidental factor,

such as hemorrhage, or intestinal perforation destroy the individual, the patient must recover. The disease, however, is long drawn out, and the patient is left much weakened and exhausted.

The prolonged incubation period following the introduction of bacteria into the tissues, and the development of the reaction stage, can be avoided by the previous sensitization of the individual to the typhoid protein. This form of prophylactic vaccination, has been the one most commonly employed, and hitherto, most successful. By means of parenteral injections of typhoid protein, the tissues of the individual are rendered hypersensitive to the protein. When, subsequently, bacteria invade the tissues of such an individual, they are immediately subjected to the action of the antibody, and either dissolution of the bacterial cell bodies is accomplished by the antibodies alone, or, through the production of an irritant substance, phagocytic cells are induced to take up and destroy the bacilli. In this way the small number of the bacteria which enter the tissues, are overcome with a minimum expenditure of energy on the part of the body, and without the exhibition of symptoms of tissue irritation.

Prophylactic Employment of Vaccines

If the animal body be hypersensitive to the bacterioprotein, and if other exhausting factors, such as starvation, loss of sleep, intercurrent disease, overwork, or prolonged chilling, have not overcome the tissue capacity for reaction, or mechanical, thermal, or chemical injury of the tissues focally has not impaired their vitality, bacterial cell bodies, when introduced into the tissues, are immediately destroyed. If the dose of infecting microorganisms be not sufficiently large to exhaust the first order bodies, the individual bacterial cells are so acted upon by these antibodies that they are rendered irritant and phagocytosis is stimulated.

The clinical immunologist's aim in the prophylactic employment of vaccines is, in fact, the development of the hyper-

sensitive state on the part of the tissues against the microorganism employed in the injections.[13]

It is of importance that it be recognized that it is against those bacteria to which the human tissues are but relatively infrequently exposed, that the employment for prophylactic purposes of bacterioprotein injections has been most successful.

Rabies

After Jenner's introduction of purposeful infection of the human tissues by the virus of cowpox[14] in order that the individual might be protected against smallpox, the first form of active immunization to be artificially employed was that instituted by Pasteur in the prophylaxis of rabies. This eminent observer discovered that it was possible by passage of rabies virus through rabbits to obtain a strain of uniform potency. This he called the fixed virus. He also discovered that by means of the injection of graduated doses of this virus, as contained in the spinal cords of infected rabbits, it was possible to protect animals and individuals against the onset of hydrophobia. For this purpose, the cords were dried for a variable length of time over hydroxide and, for the purpose of treatment, an emulsion of the dried cords was made.

By means of the passage of natural or "street" virus through rabbits in the production of fixed virus, there is noted a tendency to produce the paralytic rather than the violent form of the disease. There is also a reduction in infectivity and shortening of the incubation period.

The table on page 292 is quoted from Stimson.

This table indicates the amount of material in terms of spinal cord, plus virus, which is injected in the prophylactic treatment of the individual who is suspected, or known, to have been infected with rabies.

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[13]Although the author lays stress upon the importance of hypersensitiveness in the prevention of infection, he is familiar with the fact that under exceptional circumstances complete destruction of bacteria may be accomplished by serum bodies alone.

[14]As the principles underlying vaccinia vaccination have been extensively discussed in the chapter on the allergic reaction, this most important of all forms of prophylactic immunization is omitted in this chapter.

The following objections to the employment of rabies virus are quoted from Stimson.[15] "The administration of rabies vaccine is not entirely devoid of danger to the recipient. In a very small proportion of those receiving it a paralytic condition develops which may fairly be attributed to the treatment *per se* or to a combination of this with a peculiar susceptibility of the patient. About one-fourth of these cases end fatally, a complete recovery usually occurring in the remainder. Exposure to cold or chilling, fatigue, and the use of alcohol apparently predispose to the development of this condition, but cases are seen in which none of these factors have been operative. Other objectionable results of the treatment amount only to minor discomforts and inconveniences."

TREATMENT FOR ADULT

DAY OF TREATMENT	CORD DRIED DAYS	AMOUNT OF CORD CM.	DAY OF TREATMENT	CORD DRIED DAYS	AMOUNT OF CORD CM.
First	6	1	Twelfth	3	0.5
Second	5	1	Thirteenth	3	0.5
Third	4	1	Fourteenth	2	0.5
Fourth	3	0.5	Fifteenth	2	0.5
Fifth	3	0.5	Sixteenth	4	0.5
Sixth	2	0.5	Seventeenth	3	0.5
Seventh	2	0.5	Eighteenth	2	0.5
Eighth	1	0.5	Nineteenth	3	0.5
Ninth	5	0.5	Twentieth	2	0.5
Tenth	4	0.5	Twenty-first	1	0.5
Eleventh	4	0.5			

Antityphoid Vaccinations.—Since the time that Pasteur established a successful method of inducing active artificial immunity in the treatment of rabies, the most outstanding proof of the value of prophylactic parenteral administration of dead or attenuated bacteria, in order to confer immunity upon the injected individual, has been that of the employment of vaccination with typhoid and the paratyphoid bacilli during the war. All physicians are familiar with the fact that this form of injection—first employed by Wright and Leishman during the South African War,—has resulted in great diminution in the incidence of, and almost complete elimination of the

[15]Stimson: Jour. Am. Med. Assn., Jan. 22, 1921, lxxvi, No. 4.

death rate from, typhoid and paratyphoid fevers wherever adequate technic in the preparation, and administration, of the antigens has been used.

A satisfactory technic is the employment, upon three successive occasions, of a mixed vaccine containing in each cubic centimeter, 1,000,000,000 typhoid bacilli, and 500,000,000 each of the paratyphoid bacilli—Alpha and Beta. The first dose consists of 0.5 c.c. of the mixed vaccine, and at intervals of one week, two further doses are administered, each of 1 c.c. It is perhaps impossible to state the length of time during which absolute immunity may be expected by such a course of treatment; it is surely useful for many years.

An accidental experiment, reported by Grant[16] is of considerable interest in this connection. A laboratory worker, who had never had typhoid fever, but who had been injected with the triple typhoid vaccine, received a massive dose of living typhoid bacilli. He sucked up a quantity of about 0.5 c.c. of a culture suspension. Four days later he suffered from headache, followed by malaise, and on the eighth day, headache and weakness. On the twelfth day, after infection, Bacillus typhosus was present in his stools, but by the fifteenth day they had disappeared and were not again discovered. This case demonstrates that in certain cases, at least, typhoid vaccination protects against even massive infection. At no time could microorganisms be cultivated from his blood.

Physicians should have no hesitation in recommending to their patients that they should subject themselves to a course of prophylactic treatment with typhoid and paratyphoid vaccine.

Prophylactic Immunization Against Diphtheria

A large proportion, probably the majority, of individuals are immune to diphtheria. Since the organization of the test devised by Schick, it has been possible to determine safely and with comparatively little inconvenience to the tested individual, whether or not he or she is naturally immune. A number

[16]Grant, B. C.: Jour. Am. Med. Assn., 1921, lxxvi, 514.

of observers, more particularly Park, have recommended that in public institutions and schools, those children who are found by Schick's test to be subject to intoxication by diphtheria toxin, should be treated with the purpose of provoking an active immunity.

The Schick test is performed by injecting intradermically $\frac{1}{50}$ of one M.L.D. of a special toxin contained in 0.2 c.c. of salt solution. The injection is made into the skin on the flexor surface of the arm or forearm and a control injection is made of the same quantity of broth which has been heated to 75° C., and is consequently not toxic. If there is no antitoxin present in the tissues a positive reaction is obtained. The cells are injured and an inflammatory reaction which is characterized by redness and swelling appears in from 12 to 24 hours; and the reaction lasts for about 48 hours. Such a positive reaction indicates that the child is susceptible to diphtheria.

As early as 1905 Park noted that guinea pigs and horses treated with neutral mixtures of toxin and antitoxin produced immune sera of moderate potency. From this observation has developed the method which has been employed in the active prophylactic immunization of human cases. Neutral mixtures of toxin and antitoxin are administered. The injection is given subcutaneously and is repeated three times at intervals of one week. Those who have employed the method on a large scale are unanimous in their verdict as to its usefulness in eliminating diphtheria from public institutions where children are housed.

Park[17] reports experiments of the New York Department of Health in the use of Schick test and of toxin-antitoxin injections in the prevention of diphtheria. The Department has under observation and indexed 180,000 children; of these 90,000 have been Schick tested. Of these 90,000, about 60,000 originally gave a negative test. After injection of toxin-antitoxin mixtures, 20,000 of those which had been previously positive to the Schick test were negative. The remaining 10,000 either remained positive or were not retested.

[17]Park: New York State Jour. Med., 1923, xxiii, 1.

In a three months' period there occurred fifty-four cases of diphtheria among 90,000 untreated school children, whereas in 90,000, who had been tested and if positive treated, there were only twelve cases. It is thus seen that among untreated children diphtheria developed four and one-half times as frequently as in tested children. The highest incidences of the disease among the tested children was among 1,800 who, in spite of injections, developed an insufficient amount of antitoxin to prevent a positive Schick reaction. This result, Park points out, emphasizes the necessity of a Schick test months after the completion of the injections, and reinjection of those who are still positive.

Prophylactic Vaccination Against Other Diseases.—Although in the prevention of no other disease have such strikingly successful results been attained by means of the prophylactic employment of vaccines, as in the prevention of the enteric group of fevers, those who have had experience are well nigh unanimous in their opinion that properly employed prophylactic "immunization" against cholera, bacillary dysentery, and bacillus pestis infection, has been successful.

Vaccines prepared with Bordet's bacillus of whooping cough have been employed for several years as a prophylactic agent. The results so far obtained are still open to a certain amount of doubt as to their efficacy. It would appear, however, that in those centers in which adequate dosage had been employed, the results not only prove the relationship of the bacillus to pertussis, but also that the disease can be prevented by the proper administration, parenterally, of the specific protein antigen.

In influenza the doubt which still exists as to the relationship of the Pfeiffer bacillus to this disease has perhaps rendered attempts at prophylactic vaccination inadequate on the one hand, and lacking in proof on the other. The author has had no personal experience in the use of such vaccines. In view of the fact that the bacillus itself is so extremely minute, it is not improbable that the dose of bacillary protein, which has

been commonly employed, had been too small to expect to induce a high degree of hypersensitiveness to the antigen.

It is quite possible that in but a relatively few years, it will become the custom among communities which are more highly developed, from the public health point of view, for children to receive sensitizing doses of bacterial antigens of a large group of microorganisms. In this group will be the typhoid and paratyphoid bacillus, the meningococcus, the viruses of anterior poliomyelitis, measles, scarlet fever, and the other exanthemata, mumps, pertussis, and influenza. The suggestion has already been made that since newborn infants are relatively immune to measles, they should receive purposeful inoculation with measles virus, in order that a more permanent form of protection should be induced.

The Therapeutic Employment of Vaccines

Vaccines (bacterial proteins) are employed in the treatment of infective conditions for two purposes. Their ordinary use is in order that more adequate inflammatory reactions may be induced to take place, at foci of bacterial accumulation throughout the body. As previously pointed out bacteria are permitted to live and to multiply, without adequate vascular and cellular reaction, because the tissues are not hypersensitive, or because there is present in the tissues a sufficient amount of the second order antibody to render the tissues tolerant to the irritant product of the antigen—first order antibody reaction. In such cases, as for instance, furunculosis and chronic gonorrheal vesiculitis, reactions occur only after very large numbers of the bacteria have proliferated. The purpose of vaccine administration, in this type of case, is in order that focal reactions may be induced to take place before the bacteria have accumulated in such large numbers.

If the tissues of the infected person be hypersensitive, and also tolerant, reactions at the foci of bacterial protein may be induced by exhausting the second order antibodies, by means of parenteral injections of suitable doses of the same protein antigen.

In practice the suitable dose of bacterial protein is determined by means of intradermic (subepidermal) injections of bacterial suspensions or extracts in salt solution, or other vehicle, and observation of the local reaction which ensues. If the dose introduced be too small to exhaust the available second order antibodies, no reaction other than that due to an essential toxicity of the injected material takes place.[18] For practical purposes, it may be assumed that the reaction induced at the infective focus is similar in degree to that which occurs at the point of injection of the artificially prepared material. If the dose injected be too large an excessive local reaction is provoked.

As a rule the author has found that, unless infective foci are situated in vital organs, or are multiple to an extent which might result in an overwhelming of the individual in consequence of the simultaneous discharge from all the foci of the irritating product, a reaction about the point of injection characterized by hyperemia and swelling, and measuring approximately 4 to 7 cm. in diameter, indicates a suitable dose.

The usual method of indicating the size of the dose of bacterial protein employed is in terms of millions of cell bodies. If all bacteria were of the same size, and of the same relative solubility, and if all vaccines were prepared and stored under exactly the same conditions, such a method should be satisfactory. Such, however, is not the case. Bacterial cell bodies, even of the same strain, if grown on different media, vary much in size and in thickness of ectoplasm. A dose stated in millions, therefore, may actually vary over wide limits of volume or weight of bacterial cell substance. The degree of heat[19] or concentration of chemical germicide which has been employed in devitalizing the suspension, also influences mark-

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[18]It must be borne in mind that if autolysis of bacterial suspensions has occurred, a primary toxicity may be exhibited by such a preparation, and that the local reaction following the injection of such material is not allergic in character, and does not give a true picture of the relative proportion to first and second order antibodies in the body fluids of the injected individual. It is essential in the employment of vaccines that such bacterial preparations should not be employed. Again certain bacteria produce a true toxin which occasionally obscures the information ordinarily derived from allergic reactions, e. g., Schick reaction.

[19]More useful vaccines have been obtained by the author if germicides, e. g., carbolic acid, are employed to kill the bacterium than if heat is used.

edly the solubility and, consequently, the antigenic value of the vaccine.

When the suitable dose of vaccine has been determined by means of the intradermic reaction, subsequent administrations are injected subcutaneously. In general, vaccines should not be repeated more often than once in eight days. I have found that if ten days intervene between injections, the dose required to induce a given reaction at the infected foci remains practically constant. From time to time, however, during the course of vaccine treatment, it is advisable to retest the individual by means of the intradermic reaction.

Too great stress cannot be laid upon the necessity, on the part of the clinician who employs vaccines for therapeutic purposes, that he realize the alterations for the better which he hopes to induce on the part of the infected tissues. The working principle should be adopted that, so long as the clinical progress of an infective condition is satisfactory, no vaccines should be employed. If, on the other hand, it is believed that by means of the stimulation of a more severe inflammatory reaction at the infected focus, the course of the disease can be shortened with safety to the patient, vaccines become of the utmost usefulness. Obviously, it is absurd to hope that vaccines can be of value in cases of chronic suppurative osteitis consequent upon the presence of exfoliated bone fragments in the tissues. Nor is it possible to induce the absorption of the fibrous membrane covering the lung in cases of chronic infective open pneumothorax by means of injections of bacterial proteins. Vaccines have a very definite usefulness in the armamentarium of the practicing surgeon, and to a less extent of the practicing physician, in the treatment of disease. This usefulness is, however, a limited one; nor can vaccine administration be expected to perform miracles.

Vaccine Treatment of Furunculosis

Furuncules (localized abscesses) are developed by the tissues in order that focal accumulation of pyogenic cocci (almost invariably staphylococcus aureus) may be destroyed.

The tissues of the great majority of individuals in our modern urban communities are periodically subjected to invasion by such microorganisms, and in consequence are hypersensitive to staphylococcal protein. When cocci are introduced into the tissues of such an hypersensitive individual a vascular and cellular reaction immediately takes place. Unless the dose of bacteria which gains an entrance into the tissues be excessive, all of the invading microorganisms are immediately ingested by the leucocytes and destroyed. In this way a minute inflammatory focus is produced, but infection of the tissues is prevented.

An inadequate inflammatory reaction may be exhibited and the invading microorganisms consequently permitted to proliferate from one or other or a combination of the following causes.

1. The tissues may be tolerant to the bacterial protein. As a result the tissues are not irritated and consequently prompt vascular and cellular reaction does not take place.

2. The tissues may not be hypersensitive to the bacterial protein. In this event no reaction takes place until such time as the first order (anaphylactic) antibodies have been produced. During this incubation period the infecting microorganisms continue to proliferate.

3. Alexin may be depressed in consequence of starvation, loss of sleep, or exhaustion from other causes. Since the reaction between first order antibody and antigen does not result in the production of an irritant, except in the presence of alexin, depression of the latter body is accompanied by a loss of reactivity on the part of the tissues.

4. The bacteria themselves may be protected from the body fluids as a result of their growth upon, rather than within, the tissues, e.g., within the lumen of the sebaceous or other glands.

The fact that bacteria may grow upon, rather than within, the tissues, and consequently the individual remain a carrier for indefinite periods, has been referred to elsewhere. In furunculosis and carbuncle formation, massive infection of

the tissues occurs in consequence of proliferation of cocci within the glandular structures of the skin whence large numbers periodically invade the surrounding tissues.

The importance of constitutional exhaustion has been referred to elsewhere. This phenomenon is commonly exemplified among university football squads during the commencement of the season, if early practice is not properly controlled.

Lack of sufficient hypersensitiveness to staphylococci protein to induce adequate morphologic reaction is uncommon amongst city dwellers, but the susceptibility of individuals coming from agricultural districts is shown by the relatively high incidence of staphylococcal infections among medical students and probation nurses when they are first brought in contact with infective pus during their hospital training.

The most common cause of furunculosis encountered in city practice is dependent upon tolerance of the individual to staphylococcal protein.

In the treatment of recurrent furuncules, suspensions of the staphylococcus aureus are employed. I have never found that autogenous vaccines are of any special advantage as compared with good stock vaccines. Size of the dose to be employed is determined by means of the reaction which occurs when the vaccine is introduced subepidermically. Unless the vaccine which is being employed has an essential toxicity, due to autolytic products, the reaction which occurs at the site of the injection may be assumed to be comparable to that which occurs at the site of bacterial accumulations throughout the body.

As a rule a dose of from five hundred million to one thousand million cocci is employed. The injection is repeated once every seven to ten days, depending upon the clinical symptoms of the case, and upon the reaction which was obtained at the last dose.

The Treatment of Gonorrheal Infection by Means of Vaccines

It is difficult to believe that, during the acute stage of gonorrheal urethritis or vaginitis, much good can be accomplished by means of vaccine administration. Theoretically, at least,

prophylactic vaccination against the gonococcus should be of value, although I know of no experimental data which supports this opinion.

Chronic gonorrheal infections persist in consequence of the growth of gonococcal colonies upon the surface of, rather than within, the tissues. From the foci within the urethral, prostatic, or vesicular glands the cocci from time to time invade the tissues themselves. Since the individual who has suffered from a gonococcal infection for any considerable length of time, is tolerant, as well as hypersensitive, to the gonococcal protein, comparatively little effort is made on the part of the tissues to eradicate the foci. In consequence there is a tendency for the microorganism to prolong its existence indefinitely in such situations.

Metastatic gonococcal infections are hematogenous and are determined, in all probability, chiefly by the fact that minute infarcts are produced through plugging of terminal vessels by clumps of cocci. It is of importance in this connection to bear in mind that it is only in tissues which are nourished by terminal vessels such as the synovial and serous membranes, and upon the heart valves and conjunctivae, that metastatic infections ordinarily occur. Although it is apparent that the soft tissues of the patient suffering from chronic gonorrhea are easily able to destroy the causative microorganisms the latter are able to maintain their viability in the infarcted area. From such foci they invade, from time to time, the surrounding tissue in which acute inflammatory reactions are immediately set up.

The vaccine treatment of chronic gonorrheal infections is of value since, by this means, tolerance of the tissues may be exhausted and a maximum focal inflammatory reaction induced even though but few bacteria are present. Vaccine therapy cannot take the place of proper mechanical and topical treatment. In the presence of a stricture it is unreasonable to expect that focal collections of cocci can be completely eradicated, and if their eradication be not complete, relapse of the condition is sure to occur.

The most usual short-coming in vaccine treatment of gonococcal infection is that too small doses of the microorganism are employed to adequately exhaust the tolerant antibodies and so induce sufficiently severe focal inflammatory reactions. The maximum dose commensurate with safety should be employed, although it must be pointed out that, before severe reactions are induced by means of gonococcal protein injections, the case must be carefully studied in order that the involvement of tissues in which interstitial edema is not well borne may be excluded.

Tuberculin Therapy

Following the introduction by Koch in 1892 of the preparations of tubercle bacillus extracts known as T.O. and T.R.,[20]—the medical world was sanguine that a means had been placed at its disposal whereby tuberculosis could be cured, not only with certainty, but with comparative rapidity. Unfortunately, it was soon discovered that such was not the case. The early employment of the preparation recommended, as it was, by Koch, and with its mode of action appreciated even less than at the present time, resulted in its too promiscuous use and the employment of too large doses. The unsatisfactory and even injurious effects of its use resulted in tuberculoprotein preparations falling into disfavor. At the present time it is only by such clinicians as have conscientiously studied the results obtained and have been careful to employ bacterial derivatives in selected cases only, that tuberculin therapy is employed to any considerable extent.

There exist two methods whereby tuberculin may be administered, and although the effect of tuberculinization by these methods appears to be paradoxical, there are underlying principles which explain the apparent contradictions. To quote Baldwin:[21] "The use of tuberculin may produce two opposite effects * * * according to the method of administration. When used in small doses, and not increased, tuberculin

[20]T. O.—*Tuberculin Oberstand:* T. R.—*Tuberculin Residuum.*
[21]Baldwin: Yale Med. Jour., 1909, xv, 257.

maintains the sensitiveness * * * it appears to be a rational method for localized forms of tuberculosis. On the other hand, a gradual increase in the dosage leads, in favorable conditions of nutrition, to a complete loss of sensitiveness and coincident improvement in health. In pulmonary tuberculosis, at least, I feel inclined to select tuberculin immunization as the goal for treatment.''

Clinical Employment of Tuberculin Therapy.[22]—In order that our employment of any drug or bacterial preparation may be of value, it is necessary that we have at our command data relative to its effects upon animals in so far as this concerns both its usefulness and its injurious potentialities. Nor must this data consist merely in a superficial recognition of clinical effects, although it must be granted that without satisfactory end results no amount of theoretical hypothesis dealing with its probable effects will be of much avail. In order that favorable end results may be obtained and undesired sequelae avoided, the usefulness of the procedure augmented, and its deleterious effects minimized, it is essential that the minute changes in the physiology and morphology of the tissues under its stimulus be recognized.

At first sight it would appear that tuberculin injections stimulate the tuberculous host to the production of antibodies that inactivate the poisons arising from the tuberculous lesion, attack and destroy the bacilli themselves, or accomplish both these functions. The tubercle bacillus does not, however, produce an important essential toxin, nor does the serum of those who have recovered from attacks of tuberculosis contain more than a minimal quantity of bactericidal substance.

The natural defense of the body against the tubercle bacillus and the means whereby arrest and cure of disease processes produced by it are brought about, consist in the exhibition of two activities: (1) the microorganisms are destroyed by cellular phagocytosis, which in turn is dependent upon the pres-

[22]There has recently appeared a little book written by Riviere and Morland (Tuberculin Treatment, Oxford Press) in which there occurs a very clear exposition of the aims of tuberculin therapy and a detailed description of methods for its therapeutic employment. This monograph the author heartily recommends to all those interested in this subject.

ence in the body fluids of specific immune bodies (first order antibodies); (2) the focus containing the bacilli is walled off and isolated by means of the development of a dense connective tissue capsule.

Microscopic examination of tuberculous lesions of any considerable age, especially if the tissues have exhibited an ability to bring about reparative changes, reveals the fact that few blood vessels exist within the tubercle itself and that the surrounding connective tissue zone in immediate juxtaposition is practically lacking in blood vessels. Active cells either within or about the focus are, moreover, extremely scant in number. It is evident that what really has occurred in such a lesion is a more or less complete isolation of the bacilli from the surrounding tissues, so that, on the one hand the tissues are not exposed to the injurious action of the bacilli, and that, upon the other hand the bacteria are protected to a very considerable degree from the defensive properties of the body. Such a lesion is aptly termed an arrested one and is important since, although temporarily at least, the individual is free from the effects of the bacilli in the tissues, there is an ever present danger that they may commence to spread beyond their confining capsule and thus to set up more active disease.

In localized lesions, therefore, the stimulation of the tissues to increased activity, as evidenced by increased blood supply, and hence increased focal content of serum bodies and blood cells inimical to the tubercle bacilli and better nutriment for the metabolic activities of the cells *in situ,* cannot fail to increase the possibility of the total eradication of the bacilli, or a more adequate surrounding connective tissue zone production.

Tuberculin is useful in localized lesions, especially in the lymphatic glands, bones and joints, particularly after the activity of the process has been arrested by constitutional measure. So long as an adequate reaction in any tuberculous focus is taking place, it is difficult to believe that the administration of tuberculin can be useful. In those lesions which have become arrested, the normal processes of complete cure

are so extremely slow that the stimulus afforded by means of properly employed tuberculin is of value.

It is readily appreciated that two unfavorable sequelae may follow tuberculin injections if the preparation be used in too large doses or in unfavorable cases. In the first place, focal reactions in certain organs such as the brain and kidney, may prove fatal if a sufficient hyperemia be induced to interfere with the functioning of the organ as a whole, and also,—the increased toxicity conferred upon the tuberculous focus may result not simply in a useful vascular and cellular reaction but there may occur a necrosis of tissue in which the bacteria may multiply unimpeded and spread by means of the lymph and blood vessels, as well as by continuity, to more or less distant parts.

Localized necrosis or abscess formation following the employment of tuberculin is not necessarily an unfavorable development if it occurs in superficial tissues such as the skin or subcutaneous lymph nodes.

Two other types of tuberculosis may also be aided by the use of tuberculin. In certain cases the bacilli, as a result usually of an exalted second order antibody content of the blood, appear to be allowed to spread throughout the tissues with but little attempt on the part of the latter to limit the bacterial growth. In these instances, the body may be stimulated, as it were, to recognize the importance of the invader, and to adopt more adequate means for their limitation. It is noteworthy, however, that whereas in these cases a sufficiently large dose of tuberculin may do very considerable good, its repetition may produce harm.

In many cases presenting signs of toxemic fever, malaise, anorexia, loss of weight, etc., and in whom the advance of the disease process is evident—"autotoxic" (Riviere and Moreland), it is frequently possible by means of the exhibition of gradually increasing doses, repeated at short intervals, to partially exhaust the available supply of first order antibody and thus to induce a state of refractoriness or desensitization under such circumstances.

Two favorable sequelae result from such a procedure: (1) The body as a whole is freed from the constant depressing influence of the tuberculotoxic substance and is enabled, temporarily at least, to return to a more normal state of function,—the appetite improves, febrile disturbances are ameliorated, and an increase in weight is noted. (2) The tissues in the immediate vicinity of the bacilli are given an opportunity to readjust themselves so that they may be better able to withstand the encroachments of the microorganisms.

The employment of tuberculin for the purpose of inducing desensitization to tuberculoprotein in the manner just described, is much less frequently applicable in surgical practice than is the use of constant doses at long intervals, i.e., the stimulating method. The repeated injection of small gradually increasing doses of tuberculin at relatively short intervals (3-5 days) is followed by the development of the tolerant state. Pulmonary lesions are influenced more favorably by the induction of tolerance than by the stimulation of reactions. The use of this method has received its greatest stimulus on this Continent from the work of the late Dr. Trudeau and his associates at Saranac Lake. The decimal method of dosage, devised by this pioneer investigator, is usually employed.

In the author's opinion, cases which appear to be progressing favorably under symptomatic and constitutional treatment, should not be given tuberculin until such time as their lesions become inactive. Once this stage has been reached the stimulation of mild reactions at moderately long intervals will well repay the time expended thereon. Cases which are apparently not improving or arresting under careful treatment by other means should be given minute, but increasing, doses at short intervals until such time as the course of the disease is altered for the better. The employment of the bacterial preparation in these cases is similar to its use by dishonest cattle dealers in order to pass animals off as healthy while in the refractory state.

In all instances in which the physician is not able to state

clearly to himself the favorable effect which he hopes to procure from the employment of tuberculin, it should not be used.

Dosage and Interval T.R. and B.E.

Of the various preparations of tuberculin, the insoluble preparations have been used most by the author: the doses mentioned will, therefore, be in terms of T.R., so prepared that 1 c.c. represents 1 mg., dry weight of the derivatives of ground tubercle bacilli. With regard to the usefulness of other preparations, the author makes no adverse criticism, indeed it has been shown by Baldwin and Krause that practically all preparations of tubercle bacilli contain sufficient bacterial protein to induce the anaphylactic state, which, according to the hypothesis expressed in this chapter, is the fundamental principle underlying the therapeutic employment of tuberculin.

In the treatment of tuberculous processes which are accompanied by evidence of tuberculoprotein intoxication and in which it is hoped to arrest the process by inducing a stage of tolerance, doses of 0.0001 or 0.0002 should be given every two or three days, gradually increasing to 0.001 or higher, but immediately stopped should the symptoms (temperature, pulse rate, etc.) be aggravated by any dose except the first. It must be stated, however, in this connection that before instituting tuberculin therapy in these cases the patient must have been, and must continue to be kept, under a very strict regime of absolute "typhoid" rest, in so far as this concerns both physical and mental exertion.

In individuals suffering from localized lesions in which repair has so far advanced that the process has become relatively inactive, the dose of tuberculin injected must be sufficiently large to stimulate a focal inflammatory reaction. Care must be taken, however, that this be not excessive; a palpable or visible or symptomatic evidence of such reaction, unless the lesion be situated upon the surface of the body, such as the skin, iris, etc., is usually excessive. For this reason it is well

to adopt a method whereby the focal reaction is indicated by an area of inflammation at the site of injection.

White and Norman have suggested a method which is useful for this purpose. By means of the cutaneous reaction of von Pirquet, employing a known quantity of T.O., it is possible to obtain what has been called by these authors the optimum dose for therapeutic purposes. The dose which they have found to be productive, if given subcutaneously, of the most useful focal reaction without constitutional symptoms, is that which will give a reaction area of 4 mm. in diameter in from 24 to 48 hours if applied by means of the von Pirquet stylet. Thus if 1/100 c.c. of a one per cent solution gives such a mild reaction, 1/100,000 gm., or 0.1 mg. is considered the optimum dose. These authors have found that such a method of estimating the dose is reliable and that, furthermore, if the dose be repeated at intervals of two weeks the sensitiveness of the patient to tuberculin changes but little. Cushman[23] has recently reported favorably upon the use of this method in 30 cases.

The difficulty of measuring such small quantities of fluid as 1/100 c.c. and the care necessary in making the scarification, make such a method as that of White and his coworkers somewhat difficult of application. It is, however, a comparatively simple matter to introduce a measured dose of tuberculin into the superficial layers of the skin by means of a hypodermic needle. I have found that the amount of T.R. which is sufficient to produce a local reaction of 2 cm., when thus introduced, is both safe and therapeutically active. As a general rule a dose of T.R. equal to from 1/10,000 to 1/1000 of a milligram of dried tubercle products will be found to be adequate. If no reaction or an insufficient reaction be obtained by the first intradermic inoculation, the injection is repeated within two to six days when a larger dose is employed. If the original reaction is excessive,—over 2 cm. in diameters,—either a second test reaction may be performed after the lapse of two weeks or more, or the dose for subse-

[23]Cushman: Am. Jour. Med. Sc., 1913, xlvi, 213.

quent inoculations may be calculated on the basis of the degree of reaction obtained.

The Treatment of Hay Fever by Means of Pollen Extracts

Two methods may be employed in order to relieve the hay fever sufferer from symptoms of intoxication. The method more commonly employed, and the one which in general practice may be counted upon to give the most satisfactory results, is that of immunization (induction of tolerance) of the individual to the irritant product which results from the reaction of the pollen antigen with the first order antibodies.

In order that tolerance may be induced, it is advisable to commence treatment several months before the season at which pollenation of the plants responsible for the disease, occurs. By means of serial dilutions, the allergic reaction of the tissues is determined, and the first dose of protein extract injected is made to correspond to that at which a scarcely noticeable reaction occurs.

The greater amount of work upon this subject has been carried out by Chandler Walker in Boston. His method of preparing the material to be employed for the injections consists in extraction of pollen proteins in a 10 per cent alcoholic normal sodium chloride solution. After extraction for forty-eight hours the material is filtered, and the insoluble pollen constituents removed. The material recovered is considered as being composed of a dilution of pollen in the original concentration before filtering. In other words if 0.5 grams of pollen have been extracted in 500 cubic centimeters of the alcoholic saline solution and filtered the resulting extract is said to consist of a 1 to 1000 dilution. The various dilutions are made up varying from 1 to 20,000 to 1 to 100. If, when tested, a marked reaction occurs at the point where a dilution of 1 to 1000 is brought in contact with the scarified area, a moderate reaction at the point of dilution 1 to 5000, and no reaction at the point of dilution 1 to 10,000; the first dose employed consists of 2 or 3 minims of a dilution of 1 to 7,500. At weekly intervals the dose is gradually increased. In

Chandler Walker's opinion it is better to avoid reactions if possible. Over a period of three or four months gradually increasing doses are administered. The last doses of the series consist of 0.5 to 1.0 c.c. of the 1-100 dilution.

If the patient does not present himself sufficiently early in the year to undergo the prolonged course of fifteen injections, much may be done by more rapidly increasing the dosage and permitting a shorter interval (5-6 days) between injections.

Immunization of the hay fever sufferer to a specific pollen antigen is accomplished in this manner with great regularity, although it must be pointed out that, as a rule, the tolerance so induced does not persist from one year to the next. It is, therefore, necessary that treatment should be carried out each year prior to the period of onset of pollenation of the plant implicated. It is also of the utmost importance to realize that should a mistake in diagnosis be made, and treatment be instituted with a pollen extract to which the individual is not hypersensitive, no good can come to the injected individual, but on the contrary, with almost absolute certainty, will he be rendered susceptible to hay fever for many years during the season of pollenation of the individual plant which was used. It is, therefore, extremely important that not only should the history of the case be carefully enquired into, but also that the fact that the patient is actually hypersensitive to the pollen injected, be determined.

Although it is possible for persons who are brought in intimate contact with the pollen of various plants to be rendered hypersensitive to such pollen proteins, practically only three types of pollen produce troublesome hay fever. During the latter part of May and the early part of June, there are a few cases of hay fever due to June grass. Hypersensitiveness to this pollen is ordinarily unimportant and does not justify treatment. During the latter part of June and part of July, there are a moderate number of sufferers from hypersensitiveness to timothy, and closely related grasses. If such cases cannot avoid contact with the pollen, they should be treated.

The most important and disabling hay fever affections are

noted in the latter half of August, throughout September, and in the early part of October. Such cases are almost invariably due to hypersensitiveness to the protein of ragweed pollen. Since through the greater part of the continent of America this weed grows prolifically, and since the physical characteristics of the pollen are such that it may be air borne over very wide areas, it is difficult for such sufferers to protect themselves from the disease. Sufferers from hypersensitiveness to ragweed pollen may, almost invariably, be profitably treated for their affliction.

Although more favorable results are to be expected by means of a prolonged course of parenteral injections of pollen extracts in order that tolerance may be established, it is possible to so desensitize that, even during the period of pollenation of the plants responsible for the disease, the sufferer may be rendered free from symptoms. It is to be expected, moreover, that if a sufficient amount of protein antigen is injected to induce desensitization, in the course of time, although the patient must in the interval pass through a period in which hypersensitiveness is exhibited, tolerance is ultimately induced.

In order that desensitization may be accomplished, it is necessary that treatment with small doses of the pollen extract at short intervals, i.e., two or four, or more times daily, be employed. The dose injected is rapidly increased. Theoretically, the method is not without danger, and it is of the *utmost importance that none of the solution be allowed to enter a vein.* The author has been afflicted for many years with sensitization to ragweed pollen; in consequence my interest in this subject has been a very personal one. I have employed a technic which has proved useful in inducing desensitization and tolerance after the onset of the symptoms of hay fever have occurred. The method which I have found to be of value in the treatment of myself, and of a limited number of patients, has consisted in the employment of small doses of concentrated (1/100) solution of ragweed pollen. All injections have been made into the subepidermal tissues. Approximately 1 minim

of the undiluted solution, or an amount corresponding to 1 minim, has been injected at each dose. This is uniformly followed, within a few minutes, by a moderately severe focal reaction attaining a size of about 2.5 centimeters in diameter. The reaction is accompanied by slight itchiness and soreness, but not by any important feeling of malaise. Three to five doses are given at intervals of about twenty minutes on three successive days. In my opinion, adequate desensitization can usually be obtained in this way, if 1.0 c.c. of the concentrated solution be injected within three or four days.

The clinical course of the cases treated in this way has been characterized by relief from severe symptoms of hay fever, after the second day. At the end of a week, symptoms are again manifest for two or three days, when, apparently, tolerance is induced and the remainder of the season is passed through without severe symptoms.

Nonspecific Protein Therapy[24]

During the last six or seven years a method of treating certain infective conditions, more particularly the arthritides, by the injection of nonspecific proteins has been employed. As pointed out by Petersen,[25] this method seems of interest, not primarily because of the clinical results attained, but rather because it promises to exert a far-reaching influence on medical thought and theory concerning the factors that are active in recovery from disease. Throughout the course of this volume the author has, on several occasions, referred to this form of protein injection. The views expressed by a limited number of authors regarding its usefulness and manner of action are presented in this section.

The agents which have been employed include, in addition to certain chemicals and drugs, a large number of organic materials. Bacterial vaccines, such as typhoid colon and staphylococcus, have been employed for their nonspecific

[24]For a complete discussion of this subject the reader is referred to a monograph by Petersen, "Protein Therapy and Nonspecific Resistance, Macmillan and Co., 1922.

[25]Petersen: Jour. Am. Med. Assn., 1921, lxxvi, 312.

effect. Proteoses, as well as raw and boiled milk, and extracts from the tissues, all have their adherents.

Following the introduction of such agents, the tissues may react by the exhibition of a severe rigor, accompanied by a febrile reaction of 104 to 106 degrees. This is followed by a rapid fall in temperature, accompanied by sweating. Leucocytosis is provoked. At the infected foci throughout the body there is an intensification of the inflammatory reaction which later is followed by diminution which may result in the reaction being less marked than before injection.

The method has not yet become generally used, nor does it seem proper that, until more is known regarding its usefulness, its general employment should be adopted. At the present time, the best that can be said for the method is that, in occasional cases of chronic arthritis, spectacular improvement has been obtained. Again, in a number of acute inflammatory processes such as pneumonia and typhoid fever, the disease seems to have been aborted.

Miller[26] answers the question as to whether foreign protein therapy is attended with danger in the following way: "When just a sufficient amount of the protein is injected to excite a chill, it is practically free from danger." At his hospital—The Cook County Hospital, Chicago—at least 2,000 intravenous injections of typhoid vaccine, in treatment of various acute infections, have been carried out without serious consequences. The treatment has not been administered to enfeebled individuals or to those with disturbed heart action. At present it can be said only that there is some suggestion but no convincing evidence, that certain forms of sepsis may be benefited by this treatment.

In previous sections the author has referred to the useful effect of irritative protein injections in stimulating myelogenous activity with resultant peripheral leucocytosis. It has also been indicated that tolerance, which is a state much less specific in nature than is hypersensitiveness, may be exhausted by means of certain forms of proteid injection and hypersen-

[26]Miller: Jour. Am. Med. Assn., January, 1921, lxxvi, 308.

sitiveness thus made manifest. These two results may well be sufficient to justify the employment of the method, but inasmuch as similar results can usually be obtained by more specific injections, it would seem more proper that the latter technic be used.

In a review of the subject published in 1921, Petersen[27] quotes observations made by a number of authors upon the subject of increased permeability of the capillaries after nonspecific injections. Studies by von der Velden, Luithlen and Starkinstein, have justified a conclusion that such injections are followed by marked changes in the permeability of the cell membranes. They believe that it is this fundamental change that is probably at the basis of the therapeutic effect observed in nonspecific therapy.

''At first the cell membrane seems more permeable. This corresponds with the fact that there is an increase in the lymph flow, the irritability of the nerve cell is increased, and that there is a freer exchange between blood plasma and cell content—that is, sensitized cells give up their antibodies, enzymes are mobilized, thrombokinase and fibrinogen increased, and the sugar level altered. This period of increased cellular permeability corresponds with the clinical period of increased general malaise and increase of the inflammatory reaction at local foci. This phase is followed by one of diminished permeability. It is in this stage that we find the cellular resistance to intoxication increased, the threshold for nerve stimuli raised and evidences of intoxication and inflammation subsiding while the patient experiences euphoria.'' (Petersen.)

[27]Petersen: Jour. Am. Med. Assn., 1921, lxxvi, 312.

CHAPTER XXVI

THERAPEUTIC GUIDANCE OF THE ACUTE INFLAMMATORY REACTION

The acute inflammatory process is one of the chief, if not the most important, of the means at the disposal of the body for protecting itself against invasion by infective microorganisms, and for the elimination of infection. It is, at the same time, a matter of common clinical experience, that not infrequently the inflammatory reaction, *per se,* results in serious injury to the tissues and to the individual.

The surgeon is frequently called upon to interfere in order to guide the tissue reaction. It is of the utmost importance that he understand the manner in which the inflammatory reaction may be by itself productive of harm. The essential phenomena of inflammation are dilatation of vessels, exudation into the interstitial tissues of fluid and cellular accumulation. It is chiefly through an exaggeration of the second stage, namely, exudation of fluid, that harm may be accomplished. As the result of too great an amount of fluid in the interstitial tissues, the tension in such tissues may be so raised that drainage, by means of the blood and lymph vessels, from the part may be seriously interfered with, or even altogether arrested.

Under certain circumstances, it is possible for not only the efferent blood supply to be so obstructed, but even for the arterial circulation to be seriously slowed or arrested.

The primary object of vessel dilatation, and the budding of new vessels, is that an increased amount of oxygen, nutrition, and antibody and cellular content of the blood may be brought to the part. In addition the fluid content of the blood serves as a diluent for toxic products. In order that these purposes be accomplished, it is necessary that not only

an increased amount of blood be present, but that adequate circulation through the part occur. It frequently happens that as a result of increased extravascular pressure circulation is inhibited. When, through pressure in the walls of the venules or arterioles, adequate circulation has been interfered with, relative or absolute ischemia takes place. If the obstruction to blood circulation be complete, necrosis or gangrene of the tissues naturally ensues. Such an untoward effect is particularly likely to occur in narrow masses of tissue in which anastomosis of vessels is limited. This phenomenon is common as a concomitant of the acute inflammatory process affecting such tissues as the *appendix vermiformis* and the distal phalanx of the finger. When interstitial tension has become, or threatens to become, excessive, surgical interference, operative or otherwise, is indicated. Such interference should be carried out with the purpose of diminishing the extravascular pressure.

Clinical Inhibition of Inflammatory Reaction.—For purposes of preventing, or correcting, increased extravascular or interstitial tension, the surgeon has at his disposal one or more of several procedures.

(a) Rest of the inflamed part, by lessening the normal demand of the tissues for blood supply, may sufficiently diminish the peripheral pressure in the vessels that exudation may be minimized. Rest of the body conserves all the reactive properties of the body and is consequently an essential in the treatment of infective processes.

(b) Posture assists, by gravity, drainage of the interstitial spaces; the same effect can occasionally be obtained by means of compression, either by the use of an elastic stocking, or elastic bandage, in cases of varicose ulcer of the leg, or by means of the employment of adhesive strapping in the treatment of the same condition.

(c) Intermittent chilling of the part surrounding the inflammatory focus. By this means, in consequence of the induction of vascular spasm, the blood supply of the part is temporarily interfered with. During the period of dimin-

ished pressure in the afferent vessels, an opportunity is afforded for a reestablishment of more normal conditions. If the rate of drainage from the tissues remain as before, limitation of exudate into the interstitial tissue results in diminution in tension.

(d) The application of heat. By this means the vessels surrounding the inflammatory focus are dilated; insofar as this applies to the venules and lymphatic vessels its function is entirely useful. On the other hand, as clinical experience frequently exemplifies, increase in extravascular tension may be exhibited and necrosis of tissue hastened.

(e) The employment of hygroscopic agents, as for instance, concentrated salt solutions of various sorts (Wright's solution, salt packs,[1] and magnesium sulphate), or glycerine. These reagents through their ability to withdraw fluid from the tissues, are frequently potent to so diminish interstitial tension, that circulation is completely restored through the part, and more important interference is rendered unnecessary.

(f) Incision with the knife. Of all the means for diminishing interstitial tension in surgical practice, incision of the part is most important. In order that incision of indurated tense tissue may be effective, it is essential that the cut be sufficiently long and deep, to permit the discharge from the tense tissues of the extravascular fluid responsible for interference with the blood circulation. Short superficial incisions are for this purpose relatively useless, and simply serve to traumatize the already injured tissues. Obviously, unless the cutting of the tissues renders it more easy for them to wage their war upon the infecting microorganisms, the added injury caused by the scalpel can but accomplish harm. In all cases, therefore, in which incision is employed in order to restore

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[1]Although salt packs doubtless owe their usefulness in part to their activity as hygroscopic agents, the experimental work of Donaldson and Joyce (Donaldson and Joyce; Lancet, 1917, p. 445) the bacteriology of wounds treated by the method indicates that the favorable environment presented by this form of dressing for the growth of the proteolytic nonpathogenic Reading bacillus is largely responsible for the favorable results which accompany the use of the "salt pack" method. As a result of the metabolic activity of this microorganism devitalized tissue is rapidly destroyed and the tissue freed from these foci of invading microorganisms. Growth of the common progenic bacterium is, moreover, practically eliminated by the salt concentration present in the dressings.

the circulation, which has been interfered with by increased interstitial tension, any error must be made in the direction of too extensive, and too deep, rather than too limited incisions. In this connection if surgeons would habitually incise the various forms of cellulitis, more particularly of the hand, with the intention of afterwards performing secondary suture of the wound, more useful surgery would be performed, and many disabling end results would be avoided.

(g) The employment of vaccines, or bacterial proteins. If the hypothesis laid down by the author in this volume be accepted, it is obvious that the acute inflammatory reaction takes place in the tissues only if there be an interaction between the bacterial cell bodies, proteins, and the antibodies of the first order. If such an interaction takes place, the individual accumulations of bacteria become toxic foci, and the normal reaction—acute inflammation at irritant foci,—is exhibited. Continuation of the inflammatory reaction is dependent upon the presence of both of these reagents—bacterial protein and first order antibodies.

In the natural cure of infection, the inflammatory process subsides in consequence of the elimination of the invader; it is also possible to induce the arrest of the reaction by the exhaustion of the antibody. In other words, if the individual be desensitized, no reaction is exhibited at the focus of bacterial accumulation. Obviously, the arrest of the inflammatory reaction by means of desensitization through the administration of vaccines, does not bring about cure of the infection. A temporary arrest of the irritability of the focus with consequent limitation of the inflammatory reaction, may, in individual cases, result in a reestablishment, as regards circulation, of more or less normal conditions. In the author's opinion, the above is the explanation of the possible practical value of bacterial protein injections in the treatment of acute infections. In order that such desensitization may be accomplished without undue danger to the individual under treatment, it is necessary that frequent, carefully increased, doses of the antigen should be employed.

Although in my opinion, it is possible in this manner to occasionally treat inflammatory foci, in such parts of the body, such as the lungs, which have not been hitherto readily accessible for operative interference, it is a method which should be undertaken only with extreme caution, and employed only by those who are thoroughly familiar with the administration of bacterial proteins, and of the danger signals which accompany the use of any heterologous protein introduced parenterally.

Clinical Stimulation of Inflammatory Reaction.—If reaction on the part of the tissues to the presence of infection be insufficient, it may be necessary to so stimulate the tissues that a more marked reaction may be exhibited. Stimulation of the tissues for this purpose may be brought about by rendering the focus of bacterial accumulation more irritating.

Absence of adequate reaction may be due to one or other of the following causes: (a) the tissues may not be hypersensitive to the bacterial protein; (b) the tissues may be tolerant to the bacterial protein to a degree which masks their hypersensitiveness; (c) the bacteria may be located in such a situation that their cytoplasm is not exposed to the effect of whatever antibodies are present in the body fluids.

Theoretically, if the tissues be not hypersensitive, the development of the hypersensitive state may be accelerated by means of the parenteral introduction of vaccines or other preparation of bacterial protein. In practice, the only example of induction of hypersensitiveness after infection is known, or is presumed to have taken place, is in the employment of Pasteur's technic in the prophylaxis of rabies.

If the tissues be tolerant to such a degree that their hypersensitiveness is masked, and inflammatory reactions are consequently not stimulated, the second order of antibodies (tolerant antibodies) may be exhausted by means of the parenteral injection of a sufficient amount of bacterial protein. In the treatment of infectious diseases such as furunculosis, chronic gonorrheal affections, focal tuberculosis, etc., by means of

vaccines, this is the aim of the physician.[2] In place of vaccines the tissues may be induced to absorb, from the infected focus, a larger quantity of the bacterial protein than usual by means of any method which will increase the blood supply to, and through, the affected part. For this purpose we may employ active exercise or passive manipulation. A similar result may also be obtained by means of the employment of either active or passive hyperemia, according to the methods introduced by Thomas and Bier.

In those cases in which, as the result of fibrous tissue encapsulation of the infected focus, the patient has ceased to suffer from active clinical manifestations of disease, excision of the part—if this be anatomically possible—removes the danger of subsequent "lighting up" of the infected focus. Again, excision of an infected focus—even though it be moderately active—may be properly undertaken if the condition has become delimited, as for instance in the case of tuberculous infection of the knee joint.

Surgical Removal of Irritants.—If foreign bodies are present in the tissues they must be removed, if elimination of the microorganisms is to be hoped for. It makes little difference, in this connection, whether the foreign body consist of a shell fragment, a retained rubber tube, an unabsorbed ligature or suture, necrotic fascia, exfoliated bone fragment, or pus. All of these substances act as foci in which bacteria may continue to proliferate and whence the tissues in the vicinity may be reinfected. Sterilization of wounds, whether traumatic or operative, cannot be hoped for in the presence of foreign bodies. It is of no value to attempt secondary suture of wounds so long as necrotic fascia or dead bone sequestra are present. Unless the foreign bodies are removed, it makes no difference what form of adjuvant such as Dakin's solution, B.I.P.P., flavine, etc., is employed.

The essential reason for the excision and evacuation of abscesses, which are not complicated by the presence of inter-

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[2]For a more detailed consideration of the employment of vaccines for this purpose, see Chapter XXV.

stitial tension, is in order that the dead material, pus, and necrotic tissue may be removed. Obviously, therefore, the incision required in such cases need not be larger than one which suffices to permit adequate removal of the foreign body. At the same time, it is obvious that, granted all foreign body has been removed, there is little to be gained by insertion of other substances such as rubber drains, which tend by their very presence to injure the tissues. As a working rule, it can be asserted that if excessive interstitial tension be not present and if all necrotic tissue and pus pockets have been emptied, drainage in the ordinary sense of abscesses or peritoneal or joint cavities is not necessary and can only be productive of harm.

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